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ALEXANDER. Progress in the Development of a New Tomato Variety Resistant to Leaf Mold 86

BANFIELD and SMITH. Growth and Distribution of Ceratostomella ulmi in Tissues of Elm 86

BERGE, RIKER, and BALDWIN. Correlation of Pathogenicity and "Viscosity" in Cultures of Phytomonas tumefaciens 86

BLACK. Some Insect and Host Relationships of the Potato Yellow Dwarf Virus 87

BLANK. Two Types of Yellows Resistance in Wisconsin All Seasons Cabbage 87

BOYD and TAUBENHAUS. Field Control of Die Back and Black Spot 87

BOYD and TAUBENHAUS. The Effect of Certain Chemicals on the Defoliation of Rose Plants 87

BROWN and BUTLER. Inflorescence Blight of the Date Palm 88

BUCHHOLTZ. Seed Treatment as a Control for Damping Off of Alfalfa and Other Legumes 88

BUCHHOLTZ. The Occurrence of Pythiaceous Parasites at Different Soil Levels in Relation to Fallowing Practices 88

CARTER. Melanconium betulinum on Betula in Illinois 88

CHRISTENSEN and DAVIES. Does Heterocaryosis Account for the Production of Variants in Helminthosporium? 89

CLAYTON. Studies on Methods for the Measurement of Disease Resistance in N. tabacum 89

COOK. Cross Inoculation and Morphological Studies on the Peronospora Species Occurring on Chenopodium album and Spinacia oleracea 89

COOK and CALLENBACH. Comparison of the Effectiveness of Seed-treatment Materials for the Prevention of Seed and Seedling Decays in Eastern Virginia 90

COOK. Mosaic Disease of Tithonia rotundifolia 90

COOK. Phloem Necrosis in the Stripe Disease of Corn 90

DAINES. Some Principles Underlying the Fungicidal Action of Mercury in Soils 90

DAVIS. Corn Smut—Latent and Expressed 91

DUNEGAN. The Occurrence in the United States of Two Types of Teliospores of Tranzschelia pruni-spinosae 91

EDDINGS. Bacterial Wilt of Potatoes, Tomatoes, and Eggplant Controlled with Sulphur and Limestone 91

ELLIOT, MELCHERS, LEFEBVRE, and WAGNER. Pythium Root Rot of Milo 92

ENGLISH and HEALD. The Fungi Causing Decay of Pears in Washington 92

EZEKIEL, TAUBENHAUS, and FUDGE. Further Study on the Nature of Immunity of Monocotyledonous Plants to Phymatotrichum Root Rot 92

FELIX. Verticillium a Probable Cause of a Spinach Wilt in Western New York 93

FELLOWS. Effect of Take-all Lesions on the Roots, Crowns, and Culms of Wheat Plants 93

FELLOWS. Nitrogen Metabolism of Ophiobolus graminis 93
FLOR. Browning Disease of Flax in the United States 93

HARTER. Mosaic of Lima Beans 94
HENDERSON. Effect of Nutrients on Susceptibility of Tobacco Plants to Downy Mildew 94
HENDERSON. Promising Fungicides for Tobacco Downy Mildew Control 94
HOPP. The Colored Zones Associated with Decay in Trees 95
HORSFALL and ARNOLD. The Problem of Drilling Dusted Seed: Effect of Graphite 95

IVANOFF and RIKER. Genetic Types of Resistance to Bacterial Wilt of Corn 95

JOHNSON and VALLEAU. Are Tobacco Plants Affected with Mild Mosaic Susceptible to Other Strains of the Virus? 96
JOHNSON and JONES. Virus Diseases of Peas 96
JOHNSON and MILLER. Relation of Leaf Rust (Puccinia triticina) Infection to the Rate of Transpiration in Two Varieties of Wheat 96

KADOW and ANDERSON. The Relation of Zinc Sulphate to Injury from Peach and Apple Sprays in the 1935 Season 97
KEITT, BLODGETT, and MAGIE. Progress of Studies of the Epidemiology and Control of Cherry Leaf Spot 97
KEITT and NUSBAUM. Cytological Studies of the Parasitism of Two Monoconidial Isolates of Ventuira inaequalis on the Leaves of Susceptible and Resistant Apple Varieties 97
KENDRICK. A Cowpea Resistant to Fusarium Wilt and Nematode Root Knot 98
KENDRICK and SNYDER. A Vascular Fusarium Disease of Radish 98
KOEHLER. Entry of Fusarium moniliforme and Cephalosporium acremonium into Growing Corn Ears 98

LAYTON. Anthracnose-resistant Watermelons 99
LEACH. The Relation of Soil Temperature to the Development of Fusarium Wilt of Muskmelon and the Demonstration of Internal Seed Transmission 99
LEVINE, BAMBERG, and ATKINSON. Microorganisms Antibiotic or Pathogenic to Cereal Rusts 99
LINK and WILCOX. Gall Production in High and Low Carbohydrate Tomato Plants 100
LYNEIS, RIKER, and LOCKE. Comparative Physiology of Pathogenic and Non-pathogenic Crown-gall Bacteria 100

MAGIE and HORSFAL. Relative Adherence of Cuprous Oxide and other Copper Fungicides 100
MAINS. Perennial Phlox Resistant to Powdery Mildew 101
MANNS. Peach Yellows and Little Peach Studies 101
MCCALLAN and WILCOXON. The Action of Fungous Spores on Bordeaux Mixture 101
MEIER. Collecting Microorganisms from Winds above the Caribbean Sea 102
MONTEITH and REID. Control of Brown Patch in Turf by Fanning 102
MOORE. A Partial-vacuum Method for the Inoculation of Wheat and Barley with Loose Smuts 103
MOORE. Pathogenicity of Different Collections of Ustilago tritici and U. nuda 103

NAGEL. The Influence of Cercospora-infested Soil in Relation to the Epidemiology of Cercospora Leaf Spot on Sugar Beets 103

PALMITER and KEITT. Progress of Studies of Eradicant Fungicides in Relation to Apple Scab Control 103
PELTIER. Distribution and Prevalence of Ozonium Root Rot in Shelter-belt Zone of Texas 104
PINCKARD, KEITT, and RIKER. Progress of Spraying Experiments for Control of Apple Fire Blight 104
PLAKIDAS. Crown Girdle of Pear Trees 105
POOLE. Effects of Some Copper Compounds on the Control of Bacterium pruni and on the Peach Tree 105

RAMSEY. A Tuber Rot of Southern Potatoes 105
REDDY. Effect of Seed Treatment on Disease-free and Diseased Seed Corn 105
REDDY. Flax Seed Treatment. 106
RIKER, PINCKARD, and BALDWIN. Electrical Potentials Found in Studies on Phytomonas tumefaciens and Related Organisms and on Crown Gall 106
ROSEN. The Abscission of Pear and Apple Blossoms in Relation to Infection by Erwinia amylovora and Phytomonas syringae 106

SCHULTZ and RALEIGH. Acquired Resistance of Potato to Latent Mosaic 107
SCHULTZ and RALEIGH. Reaction of a Green Mountain Potato Seedling to Composite Infections of Mild and Crinkle Mosaic and Different Types of Latent Mosaic Virus 107
SMITH. Infection Studies with Sclerotinia fructicola 107
SMITH and GOLDSWORTHY. Studies on the Life Cycle and Control of Fabraea maculata on Kieffer and Garber Pears 108
STANLEY. An Improved Method for the Preparation of Crystalline Tobacco-mosaic Virus Protein 108
STUBBS. Viroses of the Garden Pea, Pisum sativum 108
SUIT. Studies on the Winter Injury of Apple Trees 109

TAUBENHAUS and BOYD. Further Studies on the Fungicidal Properties of Sulphur 109
TAUBENHAUS, EZEKIEL, and ALSTATT. Growth of Phymatotrichum omnivorum on Normal Roots and on Roots Decayed by Root Rot 109
TEHON and JACOBS. The Dayton Elm Disease 110
TILFORD. Fasciation of Sweet Peas 110
TRUE and MILLER. Dutch Elm Disease in Dead and Dying Elms 110
TULLIS and CRALLEY. Chlorosis of Rice Induced by Iron Deficiency 111
TURNER. Pathogenicity of Sclerotium rolfsii for Young Apple Trees 111

VAHEEDUDDIN. Hybridization between Sphaerotheca cruenta and Sorosporium reilianum 111
VALLEAU. A Method for Describing Strains of Tobacco Mosaic Virus 111
VINCENT and JONES. Resistance of Potato Varieties to Infection by the Veinbanding Virus 112

WALKER. Resistance to Club Root in Brassica 112
WELLHAUSEN. Histological Changes in Resistant and Susceptible Strains of Maize Infected with Phytomonas stewarti 112
WINGARD. Parasitism of the Apple Leaf Hopper, Typhlocyba pomaria 113
WRIGHT. Some Pathological Problems in Connection with the Development of the Plains Shelter-belt Project 113

YARWOOD. Downy Mildew of Hops 113

ZAUMEYER and WADE. A Pea Streak Caused by Alfalfa Mosaic 114
ZAUMEYER and WADE. Pea Mosaic and its Relationship to Other Legume Viruses 114

Progress in the Development of a New Tomato Variety Resistant to Leaf Mold. L. J. ALEXANDER.

The inability to secure either parental fruit type from crosses between individuals of the small-fruit homozygous resistant lines and individuals of the large-fruit homozygous susceptible variety, Globe, necessitated a long program of backcrossing and selection. At the present time a number of selections, still heterozygous for resistance, produce good quality fruit that appear equal in size to that of Globe and show promise of high yield. F_1 individuals resulting from crosses between homozygous resistant and homozygous susceptible parents are all resistant. Individuals of the F_2 progenies segregate for resistance into a ratio closely approaching that of 3 resistant to 1 susceptible. Progeny tests were made from 82 of these F_2 plants. These yielded 19 homozygous resistant, 42 heterozygous resistant, and 21 homozygous susceptible progenies. F_1 individuals resulting from crosses between heterozygous resistant and homozygous susceptible parents segregate for resistance into a ratio closely approaching that of 1 resistant to 1 susceptible. Progeny tests were made from 122 of these F_1 plants. Fifty-nine of the progenies were heterozygous for resistance and 63 were homozygous for susceptibility.

Growth and Distribution of Ceratostomella ulmi in Tissues of Elm. W. M. BANFIELD and A. L. SMITH.

Spores and a sparse mycelium are found in the discolored vessels of elms affected by the Dutch elm disease. Conidia are produced by the mycelium in the vessels, are distributed by the sap stream, multiply by yeast-like budding, or give rise to new hyphae. The hyphae grow through bordered pits into contiguous vessels and there release other conidia. The vascular system of the current season's growth sheath may be quickly invaded throughout all parts of the diseased tree. The mycelium in necrotic tissues mostly parallels the vertical axis of the cell lumina occupied. In sterilized inoculated wood it develops more abundantly in the ray parenchyma cells, the lumina of which commonly are choked thereby. Hyphae penetrate along the rays from ring to ring in the wood of root and stem in necrotic tissue. Hyphal strands commonly penetrate cell walls *via* bordered pits in such tissue, but usually penetrate directly by means of very constricted mycelial strands. Conidia are copiously produced in the lumina of many fibre, vessel, or ray parenchyma cells in sterilized inoculated wood. Yeast-like multiplication of the conidia chokes the lumina of some cells with spores. (Emergency Conservation Work and Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.)

Correlation of Pathogenicity and "Viscosity" in Cultures of Phytomonas tumefaciens. T. O. BERGE, A. J. RIKER, and I. L. BALDWIN.

Differences in ring pull have been detected with the duNoüy tensiometer in giant colonies of single-cell sister cultures of *Phytomonas tumefaciens.* One of these cultures is pathogenic to tomato at 27° but not at 32° C.; the other is nonpathogenic on this host. Less pull is required to separate the ring from the surface of colonies of the pathogenic strain than from colonies of the nonpathogenic strain when grown at 26° C. on various media, respectively; *e.g.*, yeast-extract, bean-extract, carrot-extract, asparagine, and nitrate agar. More ring pull for both cultures was required when they were grown at 32° C. Also in carrot-extract, tomato-extract, and bean-extract media, nonpathogenic cultures have been found to show a higher viscosity in a modified Saybolt viscosimeter than parallel pathogenic cultures. Since such differences in "viscosity" appear to be associated with the character of the bacterial gums produced by the cultures, it seems that these substances deserve attention in relation to pathogenicity.

Some Insect and Host Relationships of the Potato Yellow Dwarf Virus. L. M. BLACK.

The clover leaf hopper, *Aceratagallia sanguinolenta*, has been found to be a vector of the virus, and viruliferous insects have been collected from clover fields. Medium red clover, *Trifolium pratense*, is susceptible to the virus, and is the chief host plant of the clover leaf hopper. The leaf hoppers become able to transmit the virus about 9 days after feeding on diseased plants and are capable of harboring the virus from early November until early April. The present-known geographical range of the insect is much wider than that in which the disease is prevalent. Koch's report of transmission by *Myzus persicae* was not confirmed, although the same strain of aphid was employed. Muncie has reported transmission by *Empoasca fabae* and *Macrosiphum solanifolii*. Results with these insects and several others have been negative. The difference in vectors offers a good explanation of the contrasting field behavior of yellow dwarf, on the one hand, and leaf roll and mosaic on the other.

Two Types of Yellows Resistance in Wisconsin All Seasons Cabbage. L. M. BLANK.

Resistance in cabbage to *Fusarium conglutinans* consists of at least two types. Type A is inherited as a single dominant character. Type B is more complex genetically. In Wisconsin All Seasons single-plant lines containing either type A or type B have been established and carried through 3 generations by self pollination. In field trials, progenies containing type B vary in the amount of resistance displayed, ranging up to complete freedom from disease symptoms. In greenhouse trials over a range of soil temperatures, the 2 types of resistance may be readily distinguished. Type A remains stable over a wide range of controlled soil temperatures. Type B resistance varies in degree in different progenies and breaks down with increase in soil temperature until it is usually completely suppressed at about 24° C. At moderate greenhouse temperatures some lines carrying type B resistance show a high percentage of diseased, but mildly affected, plants. Other lines, which are fairly high in resistance in the field, show a high percentage of severely diseased plants in the greenhouse. Type B resistance is transmitted to and expressed to a certain degree in F_1 hybrids from crosses with homozygous-susceptible plants. (Cooperative investigations between the Division of Fruit and Vegetable Crops and Diseases, U. S. Department of Agriculture, and the University of Wisconsin.)

Field Control of Die Back and Black Spot. G. T. BOYD and J. J. TAUBENHAUS.

Fair control of Diplodia die back of roses was obtained by cutting off at weekly intervals all the flower buds from the plants. Better control was obtained when, in addition to the weekly disbuddings, the plants were either sprayed with Bordeaux mixture or dusted with a mixture of 10 parts of 300-mesh sulphur, 1 part monohydrated copper sulphate, and 1 part Paris Green. Neither spraying nor dusting, without disbudding, was an effective control measure. Slight control of black spot was obtained by weekly removal of flower buds. This was due probably to the greater vigor of the disbudded plants. Good control of black spot was obtained when the plants were disbudded and also sprayed with Bordeaux mixture or dusted with the sulphur mixture mentioned above.

The Effect of Certain Chemicals on the Defoliation of Rose Plants.—G. T. BOYD AND J. J. TAUBENHAUS.

Various diseases and decays frequently develop on bundled and winter-stored rose plants that fail to shed their leaves during the harvesting season. Preliminary tests were carried out in 1935 with a large number of spray materials for defoliation of such plants. Commercial lime sulphur when diluted 1: 8 with water or when used with 0.25 per cent H_2SO_4 caused severe leaf burn but no defoliation. Dilutions of arsenic pentoxide alone or in combination with H_2SO_4 caused severe injury to both foliage

and canes. Various dilutions of H_2SO_4 and $CaSO_4$ or $FeSO_4$ were more promising as defoliating agents. Prompt defoliation was secured on Paul's Scarlet Climber and President Hoover with a spray consisting of 2 lb. of either $CaSO_4$ or $FeSO_4$ in 50 gal. of a 0.25% solution of H_2SO_4. With the Talisman variety only partial defoliation resulted from the use of these materials. A commercial sticker (Aresket) was used in these combinations to assure complete coverage of foliage.

Inflorescence Blight of the Date Palm.—J. G. BROWN AND KARL D. BUTLER.

A fungus blight of the inflorescence of Iteema, Maktoom, Sayer, Khir, and seedling date palms has caused some loss in Arizona. Symptoms include water soaking and subsequent discoloration of infected parts, death of flowers, and fracture of the main stalk, which becomes so weakened that it cannot bear the increasing weight of the fruit in the case of the pistillate inflorescence. From infected, surface-sterilized tissues 2 *Fusarium* spp. were isolated: *F. moniliforme*, identified by two Fusarium specialists, rapidly decayed healthy date rachi into which it was inoculated and from which it was afterward reisolated; the other species, identified by one authority as *F. semitectum*, by the other as *F. lateritium* var. *fructigenum*, caused an almost identical blight, which, however, was slower in its action.

Seed Treatment As a Control for Damping off of Alfalfa And Other Legumes.—WALTER F. BUCHHOLTZ.

Seedling stands of alfalfa, white sweet clover, Dalea, Lespedeza, red clover, and alsike clover were improved by treating the seed with an organic mercury dust before planting in Pythium-infested soil in the greenhouse. Only small increases were obtained with alsike and red clover, and white Dutch clover did not damp off in the soil used. In the field, emerged seedling stands of alfalfa were improved 8 to 145%, depending on the degree of soil infestation. On one area, apparently nearly free of Pythium, alfalfa seedling stands were depressed 5 and 11% by seed treatment. Counts taken from a field stand of alfalfa grown on unlimed Clinton silt loam from treated seed were 50% higher than those taken from an adjacent limed area seeded with inoculated seed. Nodules were found on the roots of plants grown from both treated and inoculated seed.

The Occurrence of Pythiaceous Parasites at Different Soil Levels in Relation to Fallowing Practices.—WALTER F. BUCHHOLTZ.

The Pythiaceous fungi, parasitic on alfalfa seedlings, have been found most abundantly in the upper 3 to 5 inches of top soil of Webster silt loam, Clarion loam, and Tama silt loam. An area of Webster silt loam that had been fallow two years produced a 39% emerged stand[1] of alfalfa seedlings, while an adjacent area that had grown a crop of corn the year before and had been spring-plowed, yielded a 75% stand. When the top two and the next two inches of soil were interchanged, the fallow Webster silt loam produced an 80% stand.

Melanconium betulinum on Betula in Illinois.—J. C. CARTER.

Apparently because their vigor was greatly reduced by the drought of 1934, birches (*Betula alba* and *B. papyrifera*) have been attacked this year, in both nurseries and ornamental plantings, in widely separated sections of Illinois, by a die-back disease with which a Melanconium is uniformly associated. It appears identical with *M. betulinum* as represented by No. 1998 of Sydow's Mycotheca Germanica. Fruiting bodies

[1] Percentage stand is calculated on the basis of the average number of seedlings obtained from a similar planting on nearly Pythium-free soil.

first show as small raised places in the cortex and develop into white-tipped, stromatic acervuli. Conidia germinated freely on Difco plain, malt-extract, potato-dextrose, and corn-meal agars. However, germ tubes did not develop beyond a length of 110 μ on plain agar but on other agars good colonies developed. This fungus grows slowly in culture. On malt-extract agar growth averaged 13 mm. in 48 hours and 25 mm. in 96 hours; on potato-dextrose agar 14 mm. in 48 hours and 32 mm. in 96 hours; and on corn-meal agar 13.5 mm. in 48 hours and 27.5 mm. in 96 hours. Of 3,000 counted spores suspended in water, 34.6% had germinated after 24 hours and their germ tubes averaged 33.1 μ in length. After 48 hours 50.2% had germinated and the germ tube length averaged 35.5 μ.

Does Heterocaryosis Account for the Production of Variants in Helminthosporium?—J. J. CHRISTENSEN AND F. R. DAVIES.

Variants occur frequently in Helminthosporium. The hyphal cells, conidia, and germ tubes are multinucleate, and hyphal anastomoses between races of *Helminthosporium sativum* and other dark-spore species are common. Cultural comparisons were made of 468 conidial isolates from 154 individual conidiophores obtained from mixtures of 2 distinct races or species growing on agar drops or from barley tissue infected with 2 or more distinct races or species. All isolates from the same conidiophore, with two exceptions, were identical culturally. Two hundred sixty eight monosporous isolates selected at random from mixed colonies on agar drops resembled the parental types. From 2 to 5 hyphal tips were isolated from each of 63 germinating conidia taken from colonies giving rise to variants or from mixed colonies on agar drops. With one exception, the isolates from a single conidium were identical with the original culture. Young conidiophores are uninucleate; consequently, all nuclei in conidia produced on the same conidiophore are derivatives of a single nucleus. The results indicate that the variation in the species studied is due primarily to nuclear change rather than to heterocaryosis.

Studies on Methods for the Measurement of Disease Resistance in N. tabacum.—E. E. CLAYTON.

Extensive collections of tobacco varieties and strains are being studied for resistance to black rootrot (*Thielaviopsis basicola*), Granville wilt (*B. solanacearum*), Fusarium wilt (*Fusarium oxysporum* var. *nicotianae*), black shank (*Phytophthora parasitica* var. *nicotianae*), stem rot (*Sclerotium rolfsii*), mildew (*Peronospora tabacina*), wild fire (*B. tabacum*), black fire (*B. angulatum*) and mosaic. It has been found that the method of testing must give consideration to the type of resistance and factors that modify its expression. Inoculation technique is important with Granville wilt because both morphological and physiological resistance exist. Age of plants is an important factor in studying resistance to mildew and wild fire. Temperature conditions by favoring or retarding disease development may reveal or obscure difference in degree of resistance. Plan nutrition materially affects resistance to black fire and wild fire, and with these diseases storm effects must also be considered. By carefully conducted tests in laboratory and greenhouse it is possible to confine field work to small selected groups, with a material saving in time and expense.

Cross Inoculation and Morphological Studies on the Peronospora Species Occurring on Chenopodium album and Spinacia oleracea.—HAROLD T. COOK.

Cross inoculations and morphological studies were conducted with the *Peronospora* sp. occurring on *Chenopodium album* (lamb's quarters) and *Spinacia oleracea* (spinach) in Virginia to determine if they are identical. In the cross-inoculation tests lamb's

quarters plants were infected only by conidia from this host, and spinach plants were infected only by conidia from spinach. Morphological studies showed that the conidia, conidiophores, and oospores of the Peronospora on spinach differed from those of the Peronospora on lamb's quarters. It is concluded from this study that the 2 are distinct species and that *C. album* does not serve as a host of the spinach mildew fungus.

Comparison of the Effectiveness of Seed-treatment Materials for the Prevention of Seed and Seedling Decays in Eastern Virginia.—HAROLD T. COOK AND JOHN A. CALLENBACH.

A number of seed-treatment materials were compared for their effectiveness in the prevention of seed and seedling decays of tomatoes under greenhouse and field conditions. These treatments were supplementary to $HgCl_2$ treatment (1: 2000 for 7 minutes) for the control of seed-borne diseases. ZnO, Cu_2O, Vasco 4, and Semesan were applied as dusts and tested both in the greenhouse and field. $CuSO_4$, applied as a soak, was tested only in the field and resulted in severe injury. Vasco 4, ZnO, Cu_2O, and Semesan ranked in the order named in both greenhouse and field tests.

A Mosaic Disease of Tithonia rotundifolia.—MELVILLE T. COOK.

A single specimen of *Tithonia rotundifolia,* an introduced ornamental plant in Puerto Rico, was found showing large yellow blotches. Inoculations were made to other plants and the symptoms reproduced. Inoculations of it to tobacco and of the yellow tobacco mosaic to Tithonia gave negative results. Only one other record of a virus disease on this genus has come to the attention of the writer. That was by van der Bijl of South Africa, who reported a virus disease on *T. diversifolia* in 1931. However, Tagetes is a synonym of Tithonia; and several virus diseases have been reported on *Tagetes erecta* and *T. patula* in the United States.

Phloem Necrosis in the Stripe Disease of Corn.—MELVILLE T. COOK.

This is the third record of phloem necrosis, which is transmitted in the seed and is not abundant in Puerto Rico, probably because it causes sterility in many diseased plants. Histological studies showed phloem necrosis in every case and a necrosis in the parenchyma in a few cases. In the most severe cases there was a complete breakdown of a large number of cells. In some cases there was an increase in the number of fibrous cells. The chloroplasts in the regions of necrosis are reduced in number and size. There is no evidence of disintegration, but abundant evidence of inhibition of these chloroplasts.

Some Principles Underlying the Fungicidal Action of Mercury in Soils.—ROBERT H. DAINES.

In soils where mercurials are effective as fungicides, mercury compounds are reduced by the soil to metallic mercury, which migrates in the soil as mercury vapors. Any factor that prevents the conversion of a mercury salt to metallic mercury destroys the fungicidal effects of the mercurial. Some of these limiting factors are: The presence of mercury-precipitating ions; a soil with a high mercury-binding capacity and a soil having a high oxidation, and, conversely, a low reducing potential. Mercurials consisting of metallic mercury deposited on suitable carriers were found to give more satisfactory control of Rhizoctonia stem lesions than any of the standard mercurials when used either as a seed potato dip or soil treatment. Mercury on Bentonite was found to give excellent control in some soils, but only partial control in others. This reduction in effectiveness was found to be corrected by using amalgams of metals above mercury in the electromotive series. In the presence of the other metals the oxidation of metallic mercury is delayed and its effective period prolonged.

Corn Smut—Latent and Expressed.—GLEN N. DAVIS.

In the fall of 1934 and 1935 the leaf sheaths were stripped from corn plants exposed to natural and artificial smut infection. Small smut galls, not large enough to rupture the leaf sheaths, aggregating 39.1 and 30.7%, respectively, of the total infection, were found at the nodes. Smut mycelium may remain latent in the nodal buds for a long period resulting in late appearance of nodal infections. Field and laboratory experiments to determine if such might be the case, including 5 years' results on inoculated and noninoculated plants injured to stimulate axillary bud development show, when figured on a per-plant basis that in the noninoculated series the removal of the ears gave an increase of 24.6%, and removal of tops and ears increased by 45.1% in the number of nodal smut galls over that in the checks. In the inoculated series, removal of the ears gave an increase of 17.2% and removal of the tops an increase of 81.7% in the number of nodal galls over that in the inoculated noninjured checks. Laboratory examination of 262 axillary buds from 50 inoculated sweet-corn plants, variety Golden Bantam, at intervals of 14–67 days after inoculation showed 140 to be infected with smut.

The Occurrence in the United States of Two Types of Teliospores of Tranzschelia pruni-spinosae.—JOHN C. DUNEGAN.

Teliospores of *Tranzschelia pruni-spinosae* produced on peach leaves differ morphologically from those produced on indigenous species of the genus Prunus. The two types of teliospores are identical with the *forma discolor* and *forma typica*, respectively, previously described in Europe, but hitherto not recognized in the United States. Inoculation experiments show that the *discolor* type, occurring on the peach and other cultivated hosts, is associated with an aecial stage on *Anemone coronaria*, while the *typica* type, occurring on indigenous hosts, is associated with an aecial stage on native species of Hepatica and Anemone. Cross-inoculation experiments and other studies indicate that the two types of teliospores represent two forms of the rust. These two forms have different aecial host plants, the urediospores, though similar do not cross infect, and the teliospores are readily distinguishable. They probably represent distinct, though closely related, species but the final decision on this point is reserved until the host relations of the basidiospores have been investigated.

Bacterial Wilt of Potatoes, Tomatoes, and Eggplant Controlled with Sulphur and Limestone.—A. H. EDDINS.

Bacterial wilt (*Bacterium solanacearum*) was commercially controlled (between crops in 1935) in experimental plots of potatoes, tomatoes, and eggplant at West Tocoi, Florida, by applying sulphur to the soil in June, 1934, then following with limestone in November. The causal organism was killed or rendered nonvirulent by broadcasting 800 lb. of freshly inoculated sulphur per A., which reduced the soil reaction from pH 5 to slightly below pH 4. The soil reaction was then restored to approximately the original pH by broadcasting 3,000 lb. of dolomitic limestone per A., which induced normal growth and yield. This treatment gave an increase of 23.1 bbl. (63.5 bus.) per A. of healthy marketable potatoes of the Spaulding Rose Variety. Only 0.8% of the tubers in the treated plots were infected; while 70.4% were infected in the controls. Tomatoes yielded 1 lb. of marketable fruit per plant in the treated soil with 17.9% of the plants killed; in nontreated soil the yield was only 1.0 lb. of marketable fruit per plant with 98.8% of the plants killed. Eggplants yielded 5.2 lb. of marketable fruit per plant with 5.9% of the plants killed in the treated soil; in nontreated soil, the yield was only 0.4 lb. per plant with 70.6% of the plants killed.

Pythium Root Rot of Milo. CHARLOTTE ELLIOTT, L. E. MELCHERS, C. L. LEFEBVRE, AND F. A. WAGNER.

For the past 10 years milos in certain localities of the Southwest have been subject to a root rot that has increased in severity on infested soil until it is no longer possible to produce a crop from susceptible varieties. Diseased plants are stunted and when 10–12 inches high the leaves begin to turn yellow along their margins and at the tips. This yellowing progresses until the plant dries without heading. All of the fine roots are destroyed and the larger roots and interior of the crown show a dark red discoloration. Soil treatments with steam, formalin, or acetic acid have shown that the causal organism is soil-borne. Isolations from diseased roots have given a fungus that, on inculation under greenhouse conditions, has produced the same symptoms as those occurring on plants grown in naturally infested soil. This fungus has been identified as *Pythium arrhenomanes* the causal organism of a root rot of corn. Certain strains and hybrids of milo and Darso are susceptible to the disease. The kafirs and sorgos tested are resistant or immune. Resistant strains of susceptible milos have been developed at the Garden City station and tested there for several years and at other stations in the Southwest. (Cooperative investigations between the Kansas Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

The Fungi Causing Decay of Pears in Washington.—HARLEY ENGLISH AND F. D. HEALD.

Representative lots of pears from the various orchard sections of the state have been studied for the development of decay. From the large number of isolations made, 44 fungi, culturally different, have been obtained. Blue molds and gray molds were most frequently isolated. The different isolates are being studied to determine the species and their degree of pathognicity. The rate of decay produced by the several isolates has been tested at room temperature (68–75°F.), common storage (40–50° F.) and at cold storage (32–35° F.). Some of the isolates caused decay at the three temperatures tested, others only at the higher temperatures, while a few were contaminates that caused no decay when pure cultures were used for inoculation. The two common forms, blue mold (*Penicillium* sp.) and gray mold (*Botrytis* sp.) were used in a series of inoculations of D'Anjou and Winter Nelis held at both common and cold-storage temperatures. Gray mold caused a more rapid decay at both temperatures than blue mold, and the D'Anjou pears were more rapidly decayed than the Winter Nelis.

Further Study on the Nature of Immunity of Monocotyledonous Plants to Phymatotrichum Root Rot.—WALTER N. EZEKIEL, J. J. TAUBENHAUS, AND J. F. FUDGE.

Previous work has indicated that juices expressed from the underground portions of monocotyledonous plants that are immune from *Phymatotrichum omnivorum* root rot, contain materials that, when added to synthetic nutrient solutions, inhibit growth of the fungus. Ether extracts of such juices are invariably inhibitory. Ethyl-ether extracts are more potent than petroleum-ether extracts. The ether-soluble potent material is not precipitated by acetone. It is removed from ether solution by aqueous sodium-carbonate solution, from which it has been recovered by ether extraction after acidification. Purified fractions prepared in this way completely prevent growth of *P. omnivorum* when added to nutrient solutions in amounts which supply often less than 0.05% of plant material. Such fractions have been prepared from onion bulbs, Gladiolus corms, and cane, canna, and Hemerocallis roots. Similar fractions of juices from susceptible, dicotyledonous sweet potato, carrot, and beet roots contained lower dry weights of material and did not impede growth of *P. omnivorum*. It seems probable that immunity of monocotyledonous plants

from root rot is due, at least in part, to relatively high concentration in roots of these plants of specific ether-soluble materials of an acidic nature.

Verticillium a Probable Cause of a Spinach Wilt in Western New York.—E. L. FELIX.

A spinach wilt, characterized by yellowing, wilting, and interior discoloration of the vascular system, has been observed annually during the past 4 years in the Elba muck section of New York. Losses ranged from a trace to 10%, with an average loss of less than 1%. The constant association of a fungus agreeing in morphology and temperature relations with *Verticillium alboatrum* and the absence of Fusarium of other organisms in the roots of recently infected plants suggest the probable pathogenicity of Verticillium for spinach. Several series of greenhouse inoculations in 1933 failed to produce the disease, but conditions may not have been favorable for infection. The optimum temperature for growth of the fungus on potato-dextrose agar was between 24° and 30° C. and the maximum between 30° and 35° C.

Effect of Take-all Lesions on the Roots, Crowns, and Culms of Wheat Plants.—HURLEY FELLOWS.

For five years obesrvations were made on the interrelation of different degrees of severity of lesions on the roots, crowns, and culms of greenhouse cultures of wheat attacked by *Ophiobolus graminis.* A definite relation was found between the presence and severity of lesions on the organs under consideration. If one organ alone was diseased, it usually was the roots. Root infection was followed quickly by crown infection. Increases in the percentages of crown infection were accompanied: (1) by increases in root and culm infections, in the severity of lesions on all parts, in the loss of roots, and in the percentage of plants dead, and (2) by decreases in the average height of plants, in the percentage of culms that headed, and in the percentage of plants that tillered. All plants killed by *O. graminis* and those on which sporulation was observed were infected in roots, crowns, and culms. (Cooperative investigations between the Kansas Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

Nitrogen Metabolism of Ophiobolus graminis. HURLEY FELLOWS.

Ophiobolus graminis, the parasite causing take all of wheat, has been found to be specific in its nitrogen requirements when the nitrogen was supplied in a modified Czapek's nutrient solution. Egg albumen, casein, peptone, and nucleic acid were the only compounds that were utilized by the fungus. The nitrogen of the other materials tried, both organic and inorganic, was unavailable. This unavailability was not affected by the hydrogen-ion concentration of the medium, by the source of carbon, nor by the presence of organic or inorganic growth-promoting materials. Many plant decoctions, as well as the tissue of several plants, were favorable media for *O. graminis.* (Cooperative investigations between the Kansas Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

Browning Disease of Flax in the United States. H. H. FLOR.

Prior to 1932, *Polyspora lini,* the browning disease of flax, was known to occur in the United States only in Michigan. During the last three years it has been found in Iowa, Minnesota, North Dakota, and Oregon. A disease survey of flax fields in southern Minnesota and northern Iowa was made in the late spring of 1935. The stem-canker phase of the disease was found in 23 per cent of the fields inspected, and the percentage of plants attacked ranged from a trace to 15. The attacked plants were usually either

killed or severely stunted. Browning was found in widely scattered fields throughout the eastern half of North Dakota during the summer of 1935, but appeared to be causing but little damage. Field and greenhouse tests conducted at Fargo, N. Dak., have shown Bison to be the most susceptible of the commercial seed-flax varieties, followed in order of decreasing susceptibility by Red Wing, Linota, Buda, and Rio. The yield of Bison flax from browning-inoculated plots was reduced to 61% and the average weight per seed to 85% of that from noninoculated plots. Differences in oil content (percentage) and quality (iodine number) of seed from the severely diseased plants and that from normal plants were insignificant. (Cooperative investigations between the North Dakota Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

Mosaic of Lima Beans. L. L. HARTER.

Mosaic of Lima beans has been reported only a few times in the United States, and in no case was there indicated any considerable amount of damage to the plants or reduction in yield. During the summer of 1935, mosaic was observed in a field of Lima beans grown on the government Horticultural Field Station farm near Beltsville, Maryland. From 5 to 10% of the plants showed unmistakable mosaic symptoms resembling in some ways the mosaic of garden beans. The plants were dwarfed and the yield noticeably reduced. Inoculation experiments made by rubbing the expressed juice from infected plants on the primary leaves of Lima beans yielded as high as 100% infection. Symptoms appeared in 6–7 days after inoculation. All attempts to infect the Stringless Green Refugee bean variety were unsuccessful, showing that it is evidently different from the common bean, *Phaseolus vulgaris*, mosaic. Attempts to transfer it to *Vicia faba* gave negative results, which show that it is probably different from the mosaic of several of the common legumes, as for example, red clover, white clover and white sweet clover. Inoculations to cucumbers and tobacco produced symptoms similar to cucumber mosaic. The identity of the Lima-bean mosaic has not been definitely determined, but some evidence indicates that it may be related to the cucumber mosaic.

Effect of Nutrients on Susceptibility of Tobacco Plants to Downy Mildew. R. G. HENDERSON.

In sand-culture experiments, tobacco plants grown in a solution low in nitrogen were very susceptible to downy mildew, as were also plants grown in a solution high in potassium. But plants grown in a solution low in potassium and relatively high in nitrogen were quite resistant. In these experiments the addition of an extra supply of a nitrate salt did not increase the susceptibility of the plants, as is usually presumed. It has been noticed in outdoor plant beds that downy mildew usually appears first on the stunted plants and on plants growing around old stumps in the bed where the plant food might be deficient.

Promising Fungicides for Tobacco Downy Mildew Control. R. G. HENDERSON.

Experiments in the greenhouse and in outdoor plant beds have indicated that cuprous oxide and benzoic acid, used with cottonseed oil emulsion, are quite effective for tobacco downy-mildew control. The cuprous oxide was used at the rate of 1 pound to 100 gallons of water and the benzoic acid at the rate of ¼ pound to 100 gallons of water. One % oil emulsion was used in both cases. Linseed oil and benzoic acid was used in one instance with good results. Picric acid proved to be less effective than benzoic acid and was injurious to the foliage.

The Colored Zones Associated with Decay in Trees. HENRY HOPP.

Various polypores, subjected to controlled environmental influences in cultures, formed colored zones (black lines) whenever the mycelium was exposed concurrently to a large amount of air and to a considerable concentration of water, provided atmospheric oxygen was present. The zones consisted of brown, gnarled hyphal masses that arose from hyaline mycelium. Colored zones of *Fomes igniarius* in Populus and Fagus (12 decayed and 10 healthy trees were studied) did not enclose the longitudinal limit of decay, but occurred where the circumference of the decayed wood cylinder adjoined the surrounding band of slightly colored, noninfected wood. Here the mycelium was exposed to large concentrations of air and water, since the intracellular space of the healthy layer contained 26% to 80% water and that of the decayed wood only 7% water. The air within the decayed and healthy portions did not differ significantly in chemical composition. The band of noninfected wood, previously termed the invasion zone, was actually the peripheral layer of heartwood. Its presence, therefore, was not a criterion of fungous parasitism. The point of inoculation was deduced from the distribution of colored zones. Typical sporophores of *Fomes applanatus* formed when cultures were ventilated and exposed to 75% relative humidity.

The Problem of Drilling Dusted Seed: Effect of Graphite. JAMES G. HORSFALL and EARL L. ARNOLD.

Last spring internal force-feed grain drills sowing peas dusted with cuprous oxide were frequently choked, sometimes broken, as in the case of copper-carbonate-treated wheat. Seeding rate was reduced as much as 30 per cent, thus giving farmers the erroneous idea that the seed protectant was valueless or worse. Increased interfacial friction between the seeds, produced by separating their surfaces with a non-lubricating fungicidal dust, was responsible for the difficulties. The friction increased with dosage, with coverage as related to mixing time, with moisture content of the seed, and hence with the length of moist storage. Merely tumbling seeds in a power treater so scarifies the surface as to increase friction. Lubrication obviously is the solution. Water sprayed on is satisfactory provided it is not given time to penetrate the seed and raise the moisture content. Finely pulverized graphite (325 mesh) is better. It can be added during the treating operation, will not soak into the seed, and is so inert as not to interfere with germination or with the protective action of the chemical. Graphite has reduced the friction to normal for cuprous oxide-treated peas, spinach, and wheat, Semesan-treated cabbage, and copper carbonate-treated wheat.

Genetic Types of Resistance to Bacterial Wilt of Corn. S. S. IVANOFF and A. J. RIKER.

Bacterial-wilt-resistance studies (*Phytomonas stewarti*), based on large numbers of Golden Bantam inbreds and F_1 hybrids, various commercial crosses, open-pollinated sweet, dent, and flint corns, showed the existence of three types of resistance: vigor-correlated (vigor measured by height), lateness-correlated, and "true" resistance. The inheritance of resistance was expressed in general as follows: Hybrids produced from low-resistant inbreds showed lower degree of resistance than hybrids produced from high-resistant inbreds (the other two plant characters, vigor and lateness, for the two groups of hybrids being about the same). Hybrids produced from low-resistant inbreds showed greater resistance than either parent, the vigor of such hybrids apparently increasing their resistance. Hybrids produced from high-resistant inbreds showed a degree of resistance approximately equal to that of the more resistant parent. Hybrid resistance in general appeared to be influenced mainly by the resistant parent, the susceptible parent having little or no influence. Open-pollinated sweet-corn varieties showed approximately the

same degree of resistance as open-pollinated dent and flint varieties of the same height and lateness.

Are Tobacco Plants Affected with Mild Mosaic Susceptible to Other Strains of the Virus? E. M. JOHNSON and W. D. VALLEAU.

Tests were made with Turkish tobacco plants to determine whether the presence of mild strains of tobacco mosaic would protect them against infection by more severe strains if the latter were introduced after the former had become established in the tip leaves. Plants having 6–7 leaves were inoculated with various natural strains of mild green tobacco mosaic and when infection was systemic, with the virus of white or yellow tobacco mosaic. Mild mosaic plants, inoculated on noninoculated leaves, with white or yellow mosaic developed white or yellow mosaic in the tip leaves in 10–19 days. If inoculations were made on mild mosaic-affected leaves, white or yellow mosaic developed in 20–60 days. Comparable plants inoculated with only white or yellow mosaic developed systemic infection in 7–10 days. Symptoms of white or yellow mosaic were only slightly less conspicuous in mild mosaic plants than in those having only white or yellow mosaic. The tests indicate that protection may be afforded individual cells or groups of cells because they probably are completely occupied by the first virus, but the plant as a whole does not develop immunity.

Virus Diseases of Peas. FOLKE JOHNSON and L. K. JONES.

Common mosaic and severe mosaic of peas belonging to the "sprenkel" and "marmor" types, as described by Merkel, occur in Washington. Based upon symptomology as well as longevity, host range, and percentage transmission tests these are caused by distinct viruses. Cross inoculations have been made with the viruses from pea, alfalfa, white clover, red clover, sweet clover, alsike clover, beans, vetch, horse bean, chick pea, and lentils. It was found that all these hosts except chick pea were susceptible to infection by the severe mosaic virus, while alfalfa, alsike clover, beans, and horse bean were susceptible to infection by the common mosaic virus. Tests with 12,000 seedlings grown from seed collected from virus-infected plants showed that these viruses are rarely transmitted through the seed. Preliminary field tests with 488 varieties and strains of peas showed no varieties immune, but 48 exhibited some degree of resistance.

Relation of Leaf Rust (Puccinia triticina) Infection to the Rate of Transpiration in Two Varieties of Wheat. C. O. JOHNSTON and E. C. MILLER.

Greenhouse experiments conducted at Manhattan, Kans., proved that heavy leaf-rust infection of a susceptible wheat (Pusa No. 4, C. I. 8899) initiated during anthesis increased transpiration an average of 17% for periods of 24–48 hours. Transpiration in a resistant variety (Pusa 52 × Federation, C. I. 11764) was increased an average of 3.1%. Infection of this variety was expressed principally by flecking and necrosis, accompanied by a few minute uredia. Water-loss measurements, made from 8 a.m. to 6 p.m. and from 6 p.m. to 8 a.m., showed that heavily rusted plants of the susceptible variety transpired an average of about 78% more water at night than did rust-free control plants. Loss from infected plants during the day was slightly lower than from non-rusted controls. This apparently was due to artificially induced closing of many stomata of infected plants caused by rust appressoria. The results indicate that the greater water loss from rusted susceptible plants is traceable to increased cuticular transpiration, induced by rupturing of host tissues. During the day the relatively small cuticular transpiration was masked by the high stomatal transpiration; at night it was easily observable. In the resistant variety there was no consistent quantitative difference in transpiration by infected and

noninfected plants during day and night. (Cooperative investivations by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Kansas Agricultural Experiment Station.)

The Relation of Zinc Sulphate to Injury from Peach and Apple Sprays in the 1935 Season.—K. J. KADOW AND H. W. ANDERSON.

Comparisons of $ZnSO_4$ with other materials were made on Ben Davis apples. $ZnSO_4$ caused severe russet in about the same degree as $CuSO_4$. Neither lime nor flotation sulphur caused russet. One thousand fruits were examined in each plot except the check, which was so severely infected that only 412 apples were available at harvest. In the control of scab, lime was 20% effective, $ZnSO_4$ 66%, $CuSO_4$ 94.5%, and flotation sulphur 91.8%. $ZnSO_4$ did not materially influence codling moth control or total residue at harvest. Lead arsenate and lime, with varying amounts of $ZnSO_4$ were applied to peach trees to determine the amount of $ZnSO_4$ necessary to correct arsenical injury. Injury in all plots was much more severe than usual, due to the extremely damp season. The results showed little difference between the various $ZnSO_4$ plots. Injury was severe in the lead arsenate-lime plot. The check was in excellent condition. In laboratory chemical studies, practically complete elimination of water-soluble arsenic from the standard lead arsenate-lime spray mixture was obtained by additions of as little as $\frac{1}{2}$ lb. $ZnSO_4$ to 100 gal. of water.

Progress of Studies of the Epidemiology and Control of Cherry Leaf Spot.—G. W. KEITT, E. C. BLODGETT AND R. O. MAGIE.

Results of studies of (1) relations of time and temperature in the inoculation chamber to incidence of infection and (2) length of incubation periods at controlled temperatures are presented graphically. Comparative trials of Bordeaux mixture and lime sulphur for four years on replicated Montmorency plots indicate the importance of cumulative effects. Bordeaux was much the more efficient spray for disease control, due largely to longer duration of effectiveness per application. Little difference in size of fruit was found between plots sprayed continuously with Bordeaux and lime sulphur, respectively. In certain cases where Bordeaux and lime sulphur were alternated annually, the lime-sulphur-sprayed fruit was substantially larger than that of corresponding Bordeaux plots. Nonsprayed or lime sulphur plots that had severe leaf-spot attacks shortly before harvest bore the largest fruit. Total sugar content of such fruit tended to be less than that of the smaller fruit from Bordeaux plots, where leaf spot was controlled. However, under some conditions, Bordeaux spraying appears to lead to actual reduction in the size of fruit. In these experiments on Montmorency any advantage of lime sulphur regarding fruit size appears to have been more than offset by greater fruitfulness following suitable use of Bordeaux.

Cytological Studies of the Parasitism of Two Monoconidial Isolates of Venturia inaequalis on the Leaves of Susceptible and Resistant Apple Varieties.—G. W. KEITT AND C. J. NUSBAUM.

Leaves of suitable varieties were infected in the greenhouse and studied cytologically. In all cases a minute infection hypha penetrated directly through the cuticle, and a dendritic subcuticular mycelium developed. Later developments varied with isolate and apple variety. Isolate 22a parasitized Yellow Transparent vigorously, Fameuse moderately: 17a parasitized Fameuse vigorously, Yellow Transparent moderately. Missouri Pippin was resistant to both isolates, reactiong differently to each. In very susceptible

leaves, host cells showed little abnormality until about 10 days after infection. Then progressive depletion of plastids and cytoplasm, attended by increased vacuolation, began to appear in the upper palisade layer at the middle of the lesion. This impoverishment gradually spread throughout the area underlying the fungus, and was followed by necrosis. The fungus showed no apparent injury until the host cells had died. In moderately susceptible leaves the fungus developed less vigorously, impoverishment of the host cells was less rapid and severe, and little necrosis occurred. In resistant leaves (Missouri Pippin), fungus growth was sharply restricted. Localized epidermal necrosis occurred with isolate 22a; none with 17a. The parasitism of *Venturia inaequalis* is especially noteworthy in that these delicately balanced host-parasite relations are maintained without the fungus penetrating beyond the subcuticular position.

A Cowpea Resistant to Fusarium Wilt and Nematode Root Knot.—JAMES B. KENDRICK.

Fusarium wilt, caused by *Fusarium bulbigenum* v. *tracheiphilum* and root knot, caused by *Heterodera marioni*, are limiting factors in the production of blackeye cowpeas in California. Field trials in 1928 and 1929 showed the Virginia blackeye cowpea, *Vigna sinensis*, highly resistant to Fusarium wilt, but not adapted to California cultural practices. Hybrids from crosses between the California blackeye and the Virginia blackeye were highly resistant, but tended towards extreme vegetative vigor, irregular and light seed production, and a somewhat small pea. Repeated planting on sick soil, extensive single plant selections, and back-crossing to the susceptible California blackeye have resulted in several strains highly resistant to both Fusarium wilt and root knot with increased production, less vigorous vines, and commercially desirable seed types. Since these strains resulted from crosses between the California and Virginia blackeye varieties, the resistant types have been designated the "Calva" blackeye, given appropriate numbers, and are being distributed to representative growers.

A Vascular Fusarium Disease of Radish.—JAMES B. KENDRICK AND WILLIAM C. SNYDER.

In April, 1934, a disease resembling cabbage yellows (*Fusarium conglutinans*) was observed in a seed field of white Chinese winter radishes in San Benito County, California. Diseased plants showed yellowing and dropping of the leaves, which was frequently confined to one side of the plant, vascular discoloration, severe stunting, and often death. A Fusarium was readily isolated from the discolored vascular tissue. White Chinese winter and white icicle radishes grown in steam-sterilized soil, inoculated with the Fusarium showed over 90% infection, while early Jersey Wakefield cabbage and Jersey kale grown in the same soil showed no evidence of the disease. Long black Spanish, California mammoth white, long scarlet, China rose winter, and French breakfast varieties of radishes also showed a high degree of susceptibility under greenhouse conditions. Macroscopic and microscopic examinations of tested single-spore cultures of the Fusarium show it to be a member of section *Elegans*. Terminal and intercalary chlamydospores and microconidia are produced abundantly, and the macroconidia are of the *Elegans* type. The production of deep purplish pigmentation and occasionally dark sclerotia definitely differentiate it from the *F. conglutinans* type, according to Wollenweber's Fusarium-Monographie.

Entry of Fusarium moniliforme and Cephalosporium acremonium into Growing Corn Ears.—BENJAMIN KOEHLER.

Ears of dent corn well covered by the husks and free from visible signs of rot were dissected in five stages of development, and parts plated. In the 500 ears plated,

the possibility of entry through the shank was ruled out except in a very few questionable cases. *Fusarium moniliforme* entered all infected ears through the tip end, passed downward along the silks, then inward along the surface of the kernels, then along the bracts and pedicels, and finally entered the vascular cylinder of the cob. It had been entered in 8 per cent of the ears in the milk stage and 46 per cent of the mature ears. When ears had been attacked by earworms only at the tip, and at the same time were free from obvious mold, infection was increased to 48 and 90 per cent, respectively. *Cephalosporium acremonium* was found in the well-protected, worm-free ears almost as frequently as *F. moniliforme*, but it was not greatly augmented by injury at the ear tips. Its path of entry also was the same in at least 90 per cent of the infected ears.

Anthracnose-resistant Watermelons.—DUKE V. LAYTON.

Inoculations with *Colletotrichum lagenarium* were made on edible varieties of *Citrullus vulgaris* including the available domestic varieties and an extensive collection of foreign varieties. In 1932, anthracnose-resistant strains were obtained from seed of watermelon native to Umtali, South Rhodesia, Africa. With crosses between this strain and susceptible varieties, resistance to anthracnose was found to be dominant in the F_1, and segregated in the ratio of 3 resistant to 1 susceptible in the F_2 generation. When the F_1 progeny was back-crossed on the susceptible parent, segregation for resistance and susceptibility was 1:1. Similar results were secured with reciprocal crosses. Since the anthracnose-resistant strains are susceptible to *Fusarium niveum*, crosses have been made on wilt-resistant varieties in order to secure resistance to both diseases. Watermelon selections have been isolated from these crosses that are homozygous for resistance to both anthracnose and wilt.

The Relation of Soil Temperature to the Development of Fusarium Wilt of Muskmelon and the Demonstration of Internal Seed Transmission.—J. G. LEACH.

Unlike most Fusarium wilts, muskmelon wilt does not depend upon high soil temperatures for best development. The optimum temperature for the development of the pathogen on agar lies between 27 and 30° C. Muskmelon seeds did not germinate well in soil below 20° C., and seedlings grew best at 35° C., the highest temperature tried. Within this range the wilt was most severe at the lower soil temperatures. When a plant is killed by wilt late in the season the pathogen often invades and rots almost mature muskmelons. Seed taken from decayed fruits and surface-disinfected yielded pure cultures of the pathogen when germinated on agar. Plants grown from such seed in steamed soil were wilted in the seedling stage. The fungus enters the seed at the point of attachment to the placenta and develops in the parenchyma of the spermoderm, but does not penetrate the perisperm nor infect the embryo. If the disease occurs in the seed-producing regions, seed infection provides means for long-range dissemination.

Microorganisms Antibiotic or Pathogenic to Cereal Rusts.—M. N. LEVINE, R. H. BAMBERG, AND R. E. ATKINSON.

A bacterial parasite, first found associated with urediospores of *Puccinia graminus* isolated from garden slugs, has since been obtained from many field rust specimens. Fairly common throughout the Mississippi Valley, it also occurs eastward to Virginia and westward to Oregon. In the field it attacks stem rust more frequently than leaf rust. In the greenhouse it attacks all cereal rusts. Pycnia, aecia, and uredia may be totally destroyed. The organism, of the genus Bacillus, inhibits development of rust on both adult plants and seedlings. High relative humidity favors its rapid development. An undetermined imperfect fungus, also first found associated with slugs, envelops

rust pustules with thick, floccose hyphal wefts, at first pinkish gray, later turning black. In the greenhouse, under high relative humidity, it soon obliterates pustules but is rarely found in nature. Recently a species of Trichoderma has been found that overgrows rust pustules under bell jars in the greenhouse. Its hyphae penetrate the urediopores through the germpores. In hanging drops urediospore germination is completely inhibited, but on host plants rust infection is not prevented, though its further development is hindered. Cooperative investigations by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture and the Division of Plant Pathology and Botany, University of Minnesota.

Gall Production in High and Low Carbohydrate Tomato Plants.—GEORGE K. K. LINK AND HAZEL W. WILCOX.[1]

Tomatoes grown in quartz sand received – N and + N nutrient solutions. When the former (hard) plants showed abundant starch and no nitrate, and the latter (soft) plants abundant nitrate and no starch, 10-, 13-, and 15-week-old plants were inoculated at the top, middle, and base with uniform doses of *Phytomonas tumefaciens*. After 5 weeks, cross-section measurements were made and used to determine percentage of gall area relative to normal stem area. It was found that: 1) This percentage was larger in – N than in + N plants at each level inoculated; 2) In both – N and + N plants this percentage was greatest in the 10-, less in the 13-, and least in the 15-week-old plants; 3) In – N plants the top gall percentage was larger than the basal-gall percentage. In + N plants this difference applied to the 13- and 15-, but not to the 10-week-old plants. These showed equal percentages. The results suggest that gall production in tomato plants is favored by substances relatively abundant in – N (hard) plants and comparatively lacking in + N (soft) plants. In the presence of these substances gall proliferation is apparently proportional to the growth rate of the axis level inoculated.

Comparative Physiology of Pathogenic and Nonpathogenic Crown-Gall Bacteria.—MARY M. LYNEIS, A. J. RIKER, AND S. B. LOCKE.

Studies have been made on the comparative physiology of 2 strains of *Phytomonas tumefaciens*, which are respectively pathogenic and nonpathogenic to tomato. The strain that does not infect tomato was found to induce galls on a tobacco hybrid. Likewise, since the temperature range 28–30° C. is critical for crown-gall development on tomato, studies were made of both cultures, respectively, above and below this range. The culture that is pathogenic at 27° but not at 32° C. on tomato, induces galls at both temperatures on the tobacco hybrid. Bacteriological characters examined have included: (1) utilization of certain sources of nitrogen and of carbon, respectively, (2) production of CO_2 and of H_2S, (3) agglutination, and (4) changes in osmotic pressures in yeast-extract and host-plant-extract media. Various working hypotheses proposed for the cause of cell stimulation by *P. tumefaciens*, based on some of these characters, have not received confirmation in these studies. The culture nonpathogenic to tomato appears to grow in the tomato plant during at least the first 2 weeks approximately as well as the pathogenic culture, indicating in the early stages no bacteriostatic effect by the plant on this culture.

Relative Adherence of Cuprous Oxide and Other Copper Fungicides.—R. O. MAGIE AND JAMES G. HORSFALL.

Tests have been made during the past 2 seasons, comparing by means of curves the initial adhesiveness and resistance to weathering or adherence of several copper fungicides,

[1] Supported in part by a grant from the Rockefeller Foundation to the University of Chicago.

also the effect of the addition of emulsified cotton-seed oil. The sprays that contained equivalent Cu contents were tested on apple and cherry foliage in the orchard. Two hundred leaves per tree on each of 5 or 6 sampling dates were analyzed for copper by the Elvehjem and Lindow modification of Biazzo's method. The fungicides stood in the descending order of their ability to adhere to the leaf approximately as follows: Cu_2O plus oil, Bordeaux mixture plus oil, Bordeaux mixture, Cu_2O, copper oxychloride (KK), copper phosphate, copper-ammonia-silicate plus oil, and copper-ammonia-silicate. The addition of emulsified cotton-seed oil to Cu_2O or to the copper-ammonia-silicate spray nearly doubled the initial adhesiveness. The term "initial adhesiveness" is here applied to the ability of the spray to become attached to the leaf in contrast to "adherence," which is applied to the resistance of spray residues to weathering.

Perennial Phlox Resistant to Powdery Mildew.—E. B. MAINS.

In 1933 powdery mildew severely attacked perennial phlox in Ann Arbor. In one garden a plant, apparently volunteer, was noticeable on account of its freedom from mildew. In 1934 and 1935, this plant and seedlings apparently arising from it were studied in contrast with named varieties. The varieties Africa, Commander, E. Pritchard, Graf Zeppelin, Lilian, Marchal French, Miss Lingard, Mrs. W. Van Beuningen, Nicholas Flammel, Paladin, Rynstroom, R. P. Struthers, Thor, Von Lassburg, and Widar were very susceptible. Enchantress, Emair Macks, Leo Schlageter, Pastel, Saladin, and Milly Van Hoboken were somewhat less susceptible. Columbia mildewed slowly, finally becoming moderately infected. No mildew was evident on the resistant plant throughout the study. Of the seedlings, 4 were very susceptible, 4 moderately susceptible, 7 showed various degrees of resistance. Nine were highly resistant showing no evidence of mildew.

Peach Yellows and Little Peach Studies.—T. F. MANNS.

Of the several insects tested, the plum hopper, *Macropsis trimaculata,* is the only one found disseminating the viruses of yellows and little peach. A survey of many Eastern and Central States (Ill., Ind., Ohio, Penna., Del., Md., and N. J.) shows plums are the host plants, though it is found on many neglected peach and apricot trees. We have found the insect most common on the Oriental plum, *Prunus salicina;* in some cases as many as 5,000 to 20,000 hoppers per tree. We have shown that plum may carry either or both of the viruses of yellows and little peach. Some of the Oriental plum varieties may carry these viruses and live for many years and at the same time breed many hoppers and disseminate these diseases. When both viruses are budded into peach or plum at the same time, yellows symptoms are the first to appear but later the yellows is suppressed and little-peach symptoms predominate. When plum hoppers, *Macropsis trimaculata,* that had fed on trees carrying yellows or little peach, were crushed and injected by hypodermic needles into growing twigs or leaves of peach no infection resulted. The incidence of infection, was very low whether trees were exposed to many or few hoppers (100, 25, 10, or 5) carrying the viruses of either yellows or little peach; after repeating these trials 3 successive years, the percentage of infection reached only 10–15% on peach seedlings. With the Elberta variety it was 25 to 50 per cent.

The Action of Fungous Spores on Bordeaux Mixture.—S. E. A. MCCALLAN AND FRANK WILCOXON.

The conclusion that fungous excrete substance capable of rendering the copper in Bordeaux mixture soluble, has been confirmed by the use of sensitive chemical tests capable of detecting and estimating concentrations of copper of the order of 1 part in 10 millions. It was found that distilled water would dissolve copper from films of dried

1: 1 Bordeaux giving solutions containing 0.2 p.p.m. of copper. If however the Bordeaux films were treated with water in which sopres (*Sclerotinia fructicola, Botrytis paeoniae, Neurospora sitophila*) had remained overnight, with subsequent filtration, 2–5 p.p.m. of copper were found in solution, an amount sufficient to prevent germination. In obtaining the spores a vacuum technique was employed that avoided contamination with the medium on which the fungus was cultured. A sufficient amount of spore excretions was collected for chemical examination. From the lead precipitate malic acid was identified as the hydrazid by its melting point. A mixed melting point with an authentic sample showed no depression. Malic acid, even in neutral solution, was able to bring large amounts of copper into solution from dried Bordeaux. Other hydroxy acids behave similarly. It is concluded that malic, and perhaps other, acids contained in spore excretions lead to the solution of sufficient copper from Bordeaux mixture to prevent spore germination.

Collecting Microorganisms from Winds Above the Caribbean Sea. FRED C. MEIER.

Pure cultures of various species of fungi, and spores embedded in petrolatum on glass slides, were obtained by the author in July from airplanes at 87 positions over Central American waters and land areas. This study, made possible by informal cooperation between Pan American Airways, the National Geographic Society, and the United States Department of Agriculture, calls attention to the trade winds as possible agencies in the dissemination of microorganisms. Sterile plates, occasionally obtained from exposures during and after rain squalls, at altitudes between 200 and 800 feet while only a few miles to leeward of the outer islands of the Caribbean, demonstrate the effectiveness of frequent showers in reducing spread of organisms by surface winds. However, viable spores taken from air currents above the clouds show that dissemination of certain fungi may occur regardless of and ultimately aided by rainfall at lower levels. This is confirmed by cultures obtained at points along the 500 mile course over water between Jamaica and Colombia and at a point some 50 miles east of Panama. Depending on exact wind direction, material collected on these last 2 courses may have traveled at least 500 to 700 miles across open water. (Cooperative Investigations, Bureau of Plant Industry and Weather Bureau, U. S. Dept. of Agriculture, Pan American Airways, and National Geographic Society assisting.)

Control of Brown Patch in Turf by Fanning. JOHN MONTEITH, JR. and MARY E. REID.

Brown-patch of turf caused by *Rhizoctonia solani* is most prevalent and destructive during periods of excessive heat combined with high humidity and heavy rainfall. For several years it has been observed that adequate circulation of air reduces the damage from certain turf ailments, especially brown patch. Openings cut through trees and underbrush in the direction of prevailing winds have repeatedly resulted in greatly reducing brown-patch damage. Tests have been made with fans to provide air circulation over bent turf during the night. In all cases a decided checking of the disease was noted resulting in an increase in growth from 10% to 40%. The benefit from fanning is probably chiefly that of drying the grass, thereby limiting mycelial growth. Fanning reduced soil temperature from 3° to 5° F. It did not materially affect the amount of grass produced where disease was not a factor. During periods of excessive rainfall it was necessary to supplement fanning with mercury fungicides to accomplish complete control. Propeller-type fans, delivering a current of air from 2 to 12 miles per hour appear to offer practical aid in controlling brown patch on many areas of turf located in air pockets. The work further emphasized the importance of making provisions for natural air circulation over turf.

A Partial-Vacuum Method for the Inoculation of Wheat and Barley with Loose Smuts. M. B. MOORE.

An apparatus has been devised by means of which individual heads of wheat or barley are subjected to partial vacuum while completely submerged in an aqueous suspension of chlamydospores of the loose smuts and by means of which as many as 30 heads can be inoculated in one hour. A glass inoculating chamber is connected by its upper end to a hand-operated vacuum pump and fitted at its lower end with a No. 7 split-rubber stopper by means of which the wheat or barley head may be sealed in the chamber. An inoculum-supply flask is placed lower than the chamber and connected with it through a rubber tube and pinch cock. When the pinch cock is opened, suction from the pump draws inoculum into the chamber. When it is closed, operation of the pump results in alternating partial vacuum and normal pressure, which replaces the air in the florets with the spore suspension. Susceptible varieties of wheat and barley averaged 58 and 43% smut, respectively, when inoculated by this method.

Pathogenicity of Different Collections of Ustilago tritici and U. nuda. M. B. MOORE.

Spore material of *Ustilago tritici* was collected from Minnesota, North Dakota, Texas, and Mexico. Each of 12 collections was inoculated by the partial-vacuum method into 6 to 8 heads of each of 9 differential varieties of wheat. Results indicate that the 12 collections comprise 5 parasitic races. In general, individual collections inoculated into particular varieties caused either no infection or from 20 to 100% infection, making possible a very sharp and definite separation of physiologic races. The results of similar inoculations on barley were not consistent enough to permit the separation of parasitic races, but did indicate that Trebi is rather highly resistant to the 9 collections of *Ustilago nuda* used.

The Influence of Cercospora-infested Soil in Relation to the Epidemiology of Cercospora Leaf Spot on Sugar Beets. C. M. NAGEL.

Studies have been made on the growth, longevity, and pathogenicity of *Cercospora beticola* in sterile soil cultures, and with naturally infested soil obtained from the field and used in greenhouse experiments. *C. beticola* was grown on 5 types of soil: peat, basic black loam, acid black loam, neutral black loam, and black loam low in organic matter. The best mycelial growth was obtained on the peat soil. The pH value of the cultures varied, although not widely, and were maintained at a temperature of 24–26° C. The cultures were kept for 5 weeks in a closed moist chamber, to eliminate drying of the soil; then they were placed at ordinary laboratory temperatures for the remainder of the experiment. The cultures soon attained an air-dry condition and mycelial growth was checked. At the end of 27 months nutrient-agar transfers showed that the organism was still viable. When sugar-beet seedlings were directly inoculated with the soil cultures, typical disease symptoms developed. A technique was developed for isolating the pathogen directly from the soil by means of the host plant. In greenhouse experiments, when sugar beets were grown on naturally infested soil, typical symptoms developed on both cotyledonary and true leaves.

Progress of Studies of Eradicant Fungicides in Relation to Apple Scab Control.—D. H. PALMITER and G. W. KEITT.

Fall treatments of apple leaves with copper-lime-arsenite sprays were somewhat less effective than they were last season in preventing development of perithecia of *Venturia inaequalis*, due chiefly to lessened dosage and exceptionally favorable climatic conditions for perithecial development. The more effective formulae reduced the number of peri-

thecia by 95–98.4%. No serious spray injury occurred. The effect of limited ascosporic inoculum in relation to epidemiology was studied on several hundred young Wealthy and Northwestern Greening trees. Plot 1 received a copper-lime-arsenite fall spray in 1934 and was nearly free of perithecia in 1935: plot 2, about 100 yards away, received no fall spray and had a light ascosporic inoculum. Neither plot was sprayed in 1935. On the basis of counts, and taking plot 2 results as 100%, the following percentage reductions in amounts of scab were indicated for plot 1: May 31 (petal-fall), Wealthy leaves 96: June 24, Wealthy fruit 91: June 29, Wealthy leaves 89, Northwestern Greening leaves 96. On Sept. 4, 89% of Wealthy fruit of plot 1 was scab-free: plot 2 bore insufficient fruit for counts. Climatic conditions for scab development in 1935 were good.

Distribution and Prevalence of Ozonium Root Rot in Shelter-belt Zone of Texas.—GEORGE L. PELTIER.

In view of the insidious nature of the indigenous fungus *Ozonium omnivorum* the infested areas within the Shelter-belt zone extending into southern Oklahoma and Texas in advance of planting were mapped with the object of avoiding these areas or employing resistant trees. The susceptible plants employed as indicators of infested lands consisted of the more common endemic herbaceous plants, shrubs, and trees, together with annual and perennial weeds and many crop plants. The universal distribution of three susceptible weeds (nightshade, ragweeds, and lambsquarters) in virgin, pasture, waste, and cultivated lands, along railroad embankments, roadsides, fence rows, ditches, washes, banks of dry creeks and on flood plains of larger streams, as well as the presence of conidial mats, aided very materially in detecting Ozonium. A section of land was deemed infested when one or more diseased plants revealed the characteristic mycelial weft on the roots, or when conidial mats were found. On the basis of the above procedure the approximate limits of root-rot infestation were found to be south of the 34th degree of latitude and east of the 100th meridian. In the main the prevalence of root rot was much more pronounced in the better agricultural lands and in the valleys. The sharpness with which the infested and noninfested areas were delimited was especially striking. (Special investigation, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture).

Progress of Spraying Experiments for Control of Apple Fire Blight.—J. A. PINCKARD, G. W. KEITT, and A. J. RIKER.

Potentialities of spraying as a control measure for fire blight have been studied at Gays Mills, Wis., for three seasons. Applications were made throughout the period from the "green-tip" stage of bud development until early August, including sprays in bloom. Lime sulphur as used against scab was of no value against blight. Suitable programs of Bordeaux, 1: 3: 50, and copper phosphate-bentonite-lime mixture, 2: 2: 4–50, appeared in most cases to reduce the amount of blight substantially (commonly by about ½ or ¾), but did not completely control it. Blossom and post-blossom sprays were important. The effectiveness of spraying varied much with seasonal and other conditions. The programs used were ineffective under the most severe conditions encountered. On Fameuse in 1935 Bordeaux, 1: 3: 50, occasioned severe russet and the copper phosphate mixture did not control scab satisfactorily. The results thus far obtained do not warrant recommendations for practice. However, they suggest substantial possibilities for spraying as a supplement to other means of blight control. For regions requiring frequent scab sprays during the critical period for blight infection, the desirability of a material that is effective against both scab and blight is indicated.

Crown Girdle of Pear Trees.—A. G. PLAKIDAS.

For several years, a disease has been affecting the Chinese sand pears in Louisiana. It is manifest as a bark canker at or near the ground line. The canker spreads irregularly, but more rapidly up and down the trunk, sometimes extending to the crotch and downward to the main roots, eventually girdling and killing the tree. In later stages of the disease, a white basidiomycetous fungus usually appears on the affected part of trunk and crown, forming white plaques between wood and bark. Inoculation tests have shown that this fungus is a secondary invader, as it fails to produce infection in living bark. Another fungus, probably *Dothiorella ribis*, is found constantly associated with the disease. Repeated inoculations with this fungus into branches and trunks of pear trees have resulted in large cankers, like those found on naturally infected trees. Observational evidence indicates a connection between freezing injury and the disease, the fungal infection starting at points of injury and spreading to sound bark. The disease has been noted also on apple and tung-oil trees. Good control has been obtained by scraping the affected bark and painting the wound with Bordeaux paste.

Effects of Some Copper Compounds on the Control of Bacterium pruni and on the Peach Tree.—R. F. POOLE.

On the basis of tests conducted during the past 3 years copper phosphate, ammonium copper silicate (Coposil), and red copper oxide (Cuprocide) have given more satisfactory reduction of *Bacterium pruni* infection than any of the many chemicals previously tested. Infection on sprayed trees was reduced more satisfactorily on fruit than on foliage. Fused bentonite sulphur, potash fish oil soap, and emulsified oils have improved the adhesive qualities of these chemicals without inducing injury or reducing the control values. Injury to foliage caused by all copper compounds used was prominent following applications before May 15. It also developed slightly late in the autumn. There was no injury throughout the summer months regardless of the strength and amounts applied.

A Tuber Rot of Southern Potatoes.—G. B. RAMSEY.

An unusual type of tuber rot was noted in South Carolina potatoes on the Chicago market in June, 1931. Yellowish brown, sunken, definitely circular lesions varying from $\frac{1}{16}$ to $\frac{3}{4}$ inch in diameter were characteristic. These spongy pocket-like lesions penetrated to a depth of $\frac{1}{8}$ to $\frac{1}{2}$ inch. The larger lesions showed concentric zonation of light and dark brown tissue on the surface, as well as in the interior. A scanty, fine, white mycelium was apparent on the surface of the larger lesions. From the interior of spongy pockets of all sizes pure cultures of an unidentified fungus have been obtained. This organism has produced neither sclerotia nor spores of any kind. The pathogenicity of the fungus has been proved by inoculation experiments.

Effects of Seed Treatment on Disease-free and Diseased Seed Corn.—C. S. REDDY.

Experiments in the greenhouse and field indicate that seed treatment may increase the yields from disease-free seed by inhibiting the action of pythiaceous fungi in the soil. Adverse conditions for germinations, such as low temperatures, were necessary in order to obtain beneficial results from seed treatment. In 1935, April 20 and 27 field plantings of disease-free seed corn were benefited 13.4 and 23.4 bushels per acre. Seed-treatment experiments in relation to rate of planting, including nearly disease-free, Diplodia-infected, and at times Basisporium-infected, and Gibberella-infected seed corn, were conducted during the period 1930 to 1934 at Ames, Iowa. Benefits from seed treatment were greater in comparisons with field stands less than the most productive ones,

being 4.4, 3.3, 2.7, and 1.1 bushels per acre, than with stands greater than the most productive ones, 2.5, – 1.3, 1.9, and – 0.7 bushels per acre. These latter differences show two mathematically significant increases and no significant decreases, which indicates that the plants from treated diseased seed were more productive than those from nontreated diseased seed. The increases could not have come from increasing the stands because the comparisons are within stands where an increase should produce a decreased yield.

Flax Seed Treatment.—C. S. REDDY.

Investigations of the causes of flax failures because of poor field stands have indicated that the seed-rotting and seedling blight organisms are not principally seed-borne but soil-borne pythiaceous fungi. Seed treatment has proved efficaceous in this case because flax becomes resistant to these attacks very soon after germination. The time of delimitation of susceptibility is more sharply marked when germination occurs in cold soil. This, in part, accounts for recommendations of early sowing and for favorable results from seed treatment only when the sowings are early. In 1935, seed of nine varieties of flax, treated with New Improved Ceresan at the rate of one-half ounce per bushel as compared with the same not treated, produced 112% more plants on heavily infested corn land and 30% more on lightly infested corn land. In two experiments on fallow, treated seed, as above, produced 74 and 132% more plants than nontreated seed. April sowings of flaxseed, involving 4 varieties and 889 replications, gave yield increases of 17 and 24%, respectively, following seed treatments with Ceresan and New Improved Ceresan, the latter costing one-fourth as much as the former.

Electrical Potentials Found in Studies on Phytomonas tumefaciens and Related Organisms and on Crown Gall.—A. J. RIKER, J. A. PINCKARD, and I. L. BALDWIN.

Investigations have been continued on the oxidation-reduction potentials induced in liquid media by certain bacteria. The single-cell cultures used included 2 cultures of *Phytomonas tumefaciens* that were pathogenic and nonpathogenic on tomato, respectively; 1 of *P. rhizogenes* and 1 of *Bacillus radiobacter*. Each was used in 3 trials with yeast-extract, 4 with tomato-stem-extract, and 4 with carrot-extract media. The readings were made with a vacuum-tube potentiometer at frequent intervals up to 14 or 28 days, depending on experimental requirements. Each of the cultures reduced the oxidation-reduction potential, respectively, in each of the media employed suggesting that such bacteria growing within injured tissue may have a reducing effect. However, no correlation appears between potentials and pathogenicity. From the standpoint of the plant, over 50 measurements made, with a platinum electrode inserted into the crown-gall tissue, showed without exception a higher potential than that on a similar electrode in contiguous stem tissue. These results indicate a greater capacity for energy exchange in crown-gall tissue than in neighboring noninfected tissue.

The Abscission of Pear and Apple Blossoms in Relation to Infection by Erwinia amylovora and Phytomonas syringae.—H. R. ROSEN.

To the extreme rainfall and cloudy days of April and May, 1935, has been attributed the relatively poor setting of fruit in the Ozarks this season. However, a careful examination of abscissed flowers gathered while still loosely clinging to the clusters or from the ground beneath the trees, showed in many instances more or less localized blackish spots on various parts of the receptacles. Out of 64 isolations from abscissed Kieffer and Garber blossoms gathered in two counties, 46 yielded pathogenic bacteria

as revealed in artificial inoculations with pure cultures. Of these 46, 29 were typical *E. amylovora* and 17 were *P. syringae*. In 5 isolations both types of bacteria were obtained. Likewise, counts made of several hundred abscissed Kieffer blossoms showed localized blackish spots in approximately 85 per cent of the blossoms. It appears that the hindrance of pollination or of setting of fruit during wet weather is not wholly a matter of adverse weather conditions.

Acquired Resistance of Potato to Latent Mosaic.—E. S. SCHULTZ AND W. P. RALEIGH.

Healthy Katahdin tuber and shoot, grafted onto latent mosaic Green Mountain, manifest necrosis of foliage and tubers (certain other potato varieties and seedlings give the same reaction to latent mosaic). Tuber progeny of the necrotic shoots develop plants manifesting different stages of necrotic symptoms; some of the shoots may be severely necrotic resulting in premature death, other shoots may show a few necrotic spots and more or less irregular light green areas. Mottled Katahdin shoots grafted onto latent mosaic Green Mountain develop mottling and mild necrosis. Likewise healthy Katahdin shoots, grafted onto mottled Katahdin, manifest mottling and a few necrotic spots. Inasmuch as the mottled and mildly necrotic Katahdin fail to develop severe necrosis on subsequent grafts on latent mosaic Green Mountain, it appears that mottled Katahdin has acquired resistance to latent mosaic. Furthermore, these reactions indicate that the latent mosaic virus harbored by the mottled Katahdin may either be attenuated or represent a weak type of the latent-mosaic virus group.

Reaction of a Green Mountain Potato Seedling to Composite Infections of Mild and Crinkle Mosaic and Different Types of Latent Mosaic Virus.—E. S. SCHULTZ AND W. P. RALEIGH.

By using different types of latent-mosaic, which can be classified according to severity of reaction on *Datura stramonium* into faint, medium, severe and virulent types, it is possible to produce different types of symptoms on Green Mountain seedlings with inoculations involving these types of latent virus and mild or crinkle-mosaic viruses. Mild-mosaic virus plus severe latent-mosaic virus causes a distinct mottling and ruffling on a Green Mountain seedling, while mild-mosaic virus plus faint latent-mosaic virus causes mottling but no ruffling. In the absence of latent-mosaic virus the mild-mosaic virus causes no mottling but slightly light green and rugose foliage on a Green Mountain seedling. These reactions indicate that mild mosaic and latent mosaic are caused by different groups of viruses and that different symptom expressions can be caused by combinations of viruses of different mosaic groups.

Infection Studies With Sclerotinia fructicola.—M. A. SMITH.

From histological studies it has been shown that infection by *Sclerotinia fructicola* of the non-brushed surfaces of the peach varieties Elberta, Hale, and Early Crawford occurs for the most part by way of hair sockets. Stomatal infection was not commonly observed. Direct infection through the cuticle of normal epidermal cells was only rarely seen. The minimum time for infection of peach fruits from which excess hair had been removed by brushing was found to be 4½ hours. Stomatal and direct infections were not common. The most common method of entry of germ tubes was by way of the hair sockets. The greater rapidity with which infection occurred on brushed fruits appeared to be correlated with the relative accessibility of the brushed-fruit surface to germinating conidia of *S. fructicola*.

Studies on the Life Cycle and Control of Fabraea maculata on Kieffer and Garber Pears.—M. A. SMITH AND M. C. GOLDSWORTHY.

Studies during the past 2 years in Missouri and Maryland indicate that the fungus, *Fabraea maculata*, found in these localities, depends principally on the overwintering of the conidial stage in bark cankers for a continuance of the life cycle rather than upon the production of ascospores in the fallen leaves. These conclusions were reached after detailed studies were made of the initial infections and of the prevalence of the ascigerous stage. Nondiseased trees did not become infected when diseased leaves were planted beneath them, while transplanted diseased nursery trees, remote from leaves of the previous year became heavily infected. Primary infections always were correlated with bark lesions containing conidia, and these infections were always found close to the original locus. The areas of infection increased very slowly, producing inverted cone-like diseased regions beneath the original cankers. Under conditions of protracted rainfall, widely scattered single infections were found on leaves and fruit. Copper phosphate mixture 2–4–2–50 throughout the season and lime sulphur 1–50, followed by Bordeaux 1–3–50, gave excellent control of leaf, fruit, and twig lesions. Lime sulphur and Bordeaux, however, caused injury to leaves and fruit, and an early fall defoliation resulted. Copper phosphate mixture caused no injury, defoliation was delayed, and greater production was observed.

An Improved Method for the Preparation of Crystalline Tobacco-mosaic Virus Protein.—W. M. STANLEY.

The highly infectious dark brown globulin fraction, obtained from extracts of mosaic-diseased Turkish tobacco plants by precipitation with ammonium sulphate, requires further purification before active protein will crystallize. Previously, purification of crude globulin was effected with about a 40% yield of active crystalline protein by the use of lead subacetate and celite. It has now been found possible to prepare crystals with a yield of about 85% by using ammoninum sulphate and celite. Purification by the new method was accomplished in 3 different operations: Firstly, about a 1 per cent solution of crude globulin containing sufficient ammonium sulphate (8–11% by weight) to cause a slight turbidity was adjusted to pH 8 and filtered through celite. Secondly, the solution at pH 8 was adjusted to about 20% ammonium sulphate and again filtered through celite. The colored materials remained in the filtrate, while active protein was retained on the celite filter cake. Thirdly, after extraction of the celite filter cake with water at pH 8, the solution obtained was adjusted to pH 4.5 and filtered through celite. Extraction of this filter cake gave an opalescent solution from which active protein was crystallized by the usual procedure.

Viroses of the Garden Pea, Pisum sativum.—MERL W. STUBBS.

Field and greenhouse studies have revealed that more than one virus may cause pea mosaic. Enation pea mosaic, *pea virus 1*, infected soybeans and all pea varieties. Symptoms were dwarfing, foliage spotting, pod distortion, and foliage enations. Pea aphids and plant extract (with or without abrasive) transmitted the virus. Four days aging *in vitro* and 1:3000 dilution inactivated the virus. Marble pea mosaic (*pea virus 2A*), Speckle pea mosaic (*pea virus 2B*), and Mild pea mosaic (*pea virus 2C*) infected white lupine and several pea varieties, excluding Perfection. Marble pea mosaic symptoms were mottling, much foliage chlorosis, dwarfing, leaf-drop, and stem discoloration. Speckle pea mosaic symptoms were speckled mottle, slight chlorosis and other symptoms less severe than those of the first. Mild pea mosaic produced only a very mild mottle. Pod distortion and

foliage enations were not produced by these 3 viruses. The 3 viruses were transmitted by pea aphids and plant extract (only with abrasive). One day aging *in vitro* and 1:1500 dilution inactivated them. All viruses studied infected crimson clover, broad bean, sweet pea, and yellow sweet clover, but none infected red clover or garden bean. No positive evidence of seed transmission was obtained.

Studies on the Winter Injury of Apple Trees.—R. F. SUIT.

Following the severe winter of 1933–34, seven types of injury were found on the apple trees in the Province of Quebec. The prevalence of crotch cankers was associated with the hardiness of the variety, the age of the tree, and the angle of the crotch. The prevalence of branch cankers was influenced by the presence of old pruning wounds, but not by the angle of the branch to the horizontal, nor by the direction of the branch. *Physalospora malorum, Schizophyllum commune, Cytospora* sp., *Phomopsis mali,* and *Nectria cinnabarina* were found as secondary organisms in crotch and branch cankers. Increasing amounts of black heart were associated with injury to the vessels and xylem parenchyma, xylem rays, cambium, phloem, and cortex, and phloem rays. Black heart of the xylem reduced the flow of water to 10–30% of normal; influenced the efficiency of new xylem formed; reduced the moisture content at mid-season, with an increase to 2.8% above normal by the end of the season; decreased the ability of cuttings to form callus; and increased the electrical conductivity of extracts. Bark splitting was observed in the spring of 1935 and was more prevalent on those varieties affected with black heart.

Further Studies on the Fungicidal Properties of Sulphur—J. J. TAUBENHAUS AND G. T. BOYD.

Germination of spores of numerous plant pathogens was inhibited by sulphur alone. In certain cases the toxicity of sulphur was increased by adding small amounts of Cuprocide (cuprous oxide) or other copper compounds. In only a few instances, sulphur alone or in combination failed to inhibit spore germination. Thus spores of *Botrytis cinerea* germinated readily on slides dusted with sulphur, copper carbonate, Cuprocide, or Cuprocide + sulphur, although germination was inhibited by thiokolized sulphur. Spores of *Myrothecium roridum* were unaffected by sulphur alone, although germination was inhibited by mixtures of sulphur and Cuprocide or monohydrated copper sulphate. Spores of *Septoria lycopersici* were not affected by sulphur or mixtures of sulphur, Paris Green, Cuprocide, or copper sulphate, but the spores of *Rhizopus nigricans, Sclerotinia americana, Guignardia bidwelli* (*Phyllosticta* stage), *Ustilago zeae, Gymosporangium* sp. from cedar apples, *Cylindrocladium scoparium, Cladosporium fulvum, Actinonema rosae, Diplodia* sp. (*Macrophoma* stage) of die back of roses, *Pestalozzia* sp. of rose canker, *Fusicladium effusum,* sclerotia of *Sclerotium rolfsii* were all inhibted and failed to germinate when dusted with sulphur or sulphur in combination with Cuprocide or other materials.

Growth of Phymatotrichum omnivorum on Normal Roots and on Roots Decayed by Root Rot.—J. J. TAUBENHAUS, W. N. EZEKIEL, AND G. E. ALTSTATT.

It is possible to recover *Phymatotrichum omnivorum* from the recently infected roots of susceptible plants, but not from roots of susceptible plants that have been completely decayed by the disease. In artificial cultures, *P. omnivorum* grew readily on fresh normal cotton or apple roots and on normal cotton roots dried for 3 weeks or longer, but grew poorly on fresh or dried infected cotton or apple roots that had been completely decayed by root rot. Addition of nutrients to completely decayed roots allowed good growth of the fungus. On decoctions from normal cotton or apple rots, *P. omnivorum* grew readily,

while growth was poor on decoctions from cotton or apple roots completely decayed by root rot. By adding nutrients to the decoctions of the completely decayed roots, good growth was obtained. These experiments indicate that the death of *P. omnivorum* on roots completely decayed by root rot is probably in part due simply to exhaustion of nutrients needed by the fungus, particularly carbohydrates, during the course of the decay.

The Dayton Elm Disease.—LEO R. TEHON AND HOMER L. JACOBS.

Since 1928 a dying natural timber and street trees of *Ulmus americana* has been observed in southern Ohio. Severe losses of valuable shade trees have occurred. The syndrome includes droop, wilting, yellowing, and falling of leaves, coupled with cambium discoloration and rotting of small roots and feeders, and cortical disintegration. No symptoms of parasitization appear in aboveground parts. Among 499 attempts at isolation from roots, 318 have remained sterile (owing partly to rigorous technique), 75 have yielded an organism tentatively referred to as Cephalosporium, and 106 have yielded in smaller numbers 11 other organisms, of which 39 were Penicillium and 20 *Fusarium solani* var. *martii*. The Cephalosporium was the only organism consistently isolated from diseased trees. Reinoculations have demonstrated that the Cephalosporium will grow on sterile elm twigs, that it will cause dwarfing and death of seedlings grown in test tubes, and that it will cause dwarfing and death of seedlings grown in test tubes, and that it will reproduce the syndrome when implanted in soil about potted elms. The fungus is limited to the phloem, cambium, and young xylem and causes death of the cambium, collapse of the phloem, and, perhaps with secondary invaders, cortical decay.

Fasciation of Sweet Peas. PAUL E. TILFORD.

Since reporting last year that a pathogenic bacterial organism had been isolated from sweet pea plants affected with the disease called fasciation, 15 single bacterial-cell isolates have been obtained, all of which are pathogenic. The morphological and physiological characteristics of these 15 single-cell isolates are essentially identical. A fasciated growth resembling that of affected sweet peas has developed on garden pea, petunia, and gypsophila plants following inoculation. The organism has been isolated from fasciated growths on geranium and chrysanthemum. Soil sterilization and seed treatment seem to be effective control measures. Treating sweet pea seed for 1 minute in alcohol followed by 20 minutes in 1: 1000 $HgCl_2$ is an effective treatment and is noninjurious to the seed.

Dutch Elm Disease in Dead and Dying Elms. R. P. TRUE and EVERETT T. MILLER.

A cooperative Dutch elm disease survey of 750 dead and dying elms was carried on inside and outside of the recognized Dutch elm disease areas in New Jersey, New York, and Connecticut from March to October, 1935. The average number of samples cultured per tree was 15. The trees varied from 3 to 60 inches in diameter at breast height, were from one-half to entirely dead, and were not known to have the Dutch elm disease. The sites of the 27 plots, which contained from 12 to 60, but usually 30 trees each, included swamps, wooded lowlands, open fields with scattered elms, yards, and streets. Within the disease zone the sites were selected to represent areas with varying numbers of confirmed Dutch elm diseased trees per square mile. The average for the 18 plots within the disease area in New Jersey, New York, and Connecticut was 9.9% diseased, while plots outside showed none of the disease. The positive findings indicate the necessity of sanitation work in connection with the Dutch Elm Disease Eradication program. Results of similar, though preliminary, surveys in Cleveland, Indianapolis, and Norfolk were negative. (Division of Forest Pathology, Bureau of Plant Industry, and Dutch Elm Disease Eradi-

cation, Bureau of Entomology and Plant Quarantine, United States Department of Agriculture.)

Chlorosis of Rice Induced by Iron Deficiency. E. C. TULLIS and E. M. CRALLEY.

In Arkansas, Louisiana, and Texas chlorosis of rice characterized by (1) bleaching (whitening, not yellowing) of leaf tips and chlorosis of other leaf parts; (2) stunting of individual leaves or entire plants, and (3) reduced yields of affected plants, has been found to be due to an iron deficiency in the plant. Differences in varietal susceptibility to chlorosis are very marked. Most commercial varieties now grown, except Rexoro and Fortuna, are susceptible. (Cooperative investigations by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Arkansas Agricultural Experiment Station.)

Pathogenicity of Sclerotium rolfsii for Young Apple Trees. THOMAS W. TURNER.

During the summer of 1934 several young apple trees, which had been set out the previous season, wilted and died rather suddenly. The plot had formerly been grown to beans, which from time to time had suffered from severe attack of Southern Blight. Tissue isolations on nutrient agar gave typical sclerotia in about 2 weeks. The fungus, carried over winter on Sabouraud's dextrose agar, was used to inoculate approximately 3-year-old plants of 10 varieties of apples, including Albemarle Pippin, Baldwin, Grimes Golden, Black Twig, Twenty Ounce, Wealthy, Winesap, Winter Banana, York Imperial, and a seedling. They were grown on a selected plot apparently free from this fungus. Inoculum was applied to injured surfaces in some cases, to intact in others, both scion and stock being employed. Almost every inoculated plant became infected as indicated by the extent of the necrotic area, whether inoculated on injured portions or not and whether on stock or scion, but many of them recovered even though the necrotic ring practically girdled the plant. Seventeen of the 44 inoculated plants were dead or dying within 49 days. The controls remained normal.

Hybridization between Sphacelotheca cruenta and Sorosporium reilianum. SYED VAHEEDUDDIN.

A fertile intergeneric hybrid was obtained by crossing monosporidial lines of *Sphacelotheca cruenta* (Kühn) Potter and *Sorosporium reilianum* (Kühn) McAlpine. Five haploid lines of *Sphacelotheca cruenta* and seven of *Sorosporium reilianum* were inoculated hypodermically, singly, and in paired combinations, into sorghum seedlings. None of the haploid lines caused infection, but 11 of 18 paired combinations produced chlorosis on leaves in 8 to 12 days. Normal sori with some characters of *Sphacelotheca cruenta* and some of *Sorosporium reilianum* developed in the inflorescences of some of the chlorotic plants. Different monosporidial combinations of the two species produced sori differing in shape and size. The echinulate hybrid chlamydospores were intermediate in size between both the parents. They germinated readily and produced promycelia, some of which bore sporidia, while others formed numerous hyphal branches but very few or no sporidia. The promycelia and sporidia were significantly larger than those of the parents, possibly due to heterosis.

A Method for Describing Strains of Tobacco Mosaic Virus. W. D. VALLEAU.

Tobacco mosaic viruses collected from tobacco and solanaceous weeds and preserved by drying, may, when tested on a necrotic-spotting and a non-necrotic-spotting variety of tobacco in the greenhouse and on tobacco in the field (for burning), be classified as shown below. Thus a virus may be characterized (but not identified) as necrotic-spotting, non-

burning, distorting, green. At least 9 of the 18 viruses here classified would be recognized as ordinary field mosaic or James Johnson's tobacco viruses 1 or 7. 1. Necrotic-spotting, non-burning, distorting—A. Spots minute: 1. Patterns yellow; 2. Patterns green. B. Spots large: 1. Patterns white. White mosaic, E. M. Johnson; 2. Patterns yellow; 3. Patterns green; 4. Patterns predominantly green, yellow in tomato. English aucuba mosaic; 5. Patterns rings. Ring mosaic, E. M. Johnson; 6. Patterns necrotic rings, in slow-growing plants. 2. Non-necrotic spotting—A. Non-burning: 1. Distorting: a. Patterns white; b. Patterns yellow. Yellow mosaic, E. M. Johnson; c. Patterns green, chlorotic and necrotic rings in Ambalema; d. Patterns green, no symptoms in Ambalema. Severe mosaics 1 and 2, E. M. Johnson; e. Patterns green, less distorting. 2. Non-distorting, mottled: a. Patterns green, several strains. Mild mosaics 1 and 2, E. M. Johnson. 3. Non-distorting, not mottled; a. Faint green rings; b. Yellow rings. B. Burning, distorting: 1. Patterns yellow; 2. Patterns green, several strains.

Resistance of Potato Varieties to Infection by the Veinbanding Virus.—C. L. VINCENT and L. K. JONES.

During the last 12 years, 1055 strains of potato seedlings have been developed and observed relative to susceptibility to infection by the veinbanding virus. All the seedling strains developed from various crosses with the following parents have proved highly susceptible to infection under field conditions: Early Six Weeks, McCormick, Late Rose, Bovee, Early Northern, Keeper, Jersey Red Skin, Irish Cobbler, American Wonder, and Burbank. Many of the strains showed little or no damage from the presence of the virus, but others exhibited extreme symptoms. Of the 420 strains having Katahdin as a parent 22 have shown a high degree of resistance. During 2 years the Katahdin has shown 33 per cent infection and the Chippewa 13 per cent in field plantings compared to 100 per cent infection in a like period of time in Gold Coin, Bliss Triumph, Irish Cobbler, Warba, and U. S. Department of Agriculture strain no. 41956. Extreme necrosis and dwarfing have been associated with the presence of the veinbanding virus in the Warba, Chippewa, and Katahdin varieties.

Resistance to Club Root in Brassica.—J. C. WALKER.

In greenhouse trials with soil from southeastern Wisconsin, naturally infested with *Plasmodiophora brassicae*, the following results were secured. In cabbage, *Brassica oleracea* var. *capitata*, only 3 plants remained free from infection out of a total of 2600 plants from 25 varieties. Shogoin turnip (*B. rapa*) yielded consistently 100% infected plants. Snowball, Purple Top Milan, and White Milan turnips were consistently free from infection. Other varieties of turnip contained varying percentages of infected plants. Most rutabaga, *B. napobrassica*, varieties tested contained small percentages or no infected plants. Great variation in resistance to infection in white mustard (*B. alba*) and in black mustard, *B. nigra*, was found when samples of each from different sections of America and Europe were compared. In each species samples with as low average infection as 16% and others as high as 90% were noted. In a cross between Snowball (resistant) and Shogoin (susceptible) turnip the F_1 hybrid plants were 441 resistant to 22 susceptible.

Histological Changes in Resistant and Susceptible Strains of Maize Infected with Phytomonas stewarti.—E. J. WELLHAUSEN.

Marked histological differences were found in the reaction of the vascular bundle to bacterial invasion in inbred lines of maize differing in degree of resistance. In certain

moderately susceptible Golden Bantam and white flint lines, infection of the protoxylem stimulated cell division and subsequent lignification in the adjacent parenchyma. A definite radial arrangement of heavily lignified cells around the point of infection was produced. The walls of newly formed tracheae were often pushed in by the pressure of these radially dividing cells. In advanced stages, the attempted formation of new tracheids at various points in this lignified area was evident. While this apparent walling off of the first point of infection was taking place, the tracheae and tracheids gradually became plugged, resulting in slow death of the plant. In very susceptible lines, rapid plugging of the xylem vessels and deterioration of the thin-wall parenchyma cells occurred. The entire bundle, including the phloem, was destroyed in extreme cases. Apparently, invasion was so rapid that destruction occurred before a counteraction of the host could be brought about. These lines completely wilted shortly after inoculation. In highly resistant lines the morphology of the bundle was not changed. Relatively few bundles showed infection, with seldom more than one or two vessels plugged or partly plugged.

Parasitism of the Apple Leaf Hopper, Typhlocyba pomaria, by Entomophthora.—S. A. WINGARD.

The leaf hopper, *Typhlocyba pomaria*, occurred in injurious numbers in Virginia apple orchards the latter part of August, 1935, and some of the growers prepared to spray for its control. Spraying, however, was prevented by almost continuous rainfall during the first week of September, after which the leaf-hopper infestation seemed to be much less severe. Upon examination, many dead leaf hoppers were found adhering to the undersurface of the apple leaves. Examination revealed that the dead leaf hoppers had been parasitized by a fungus, which, from the morphological characters of its hyphae and spores, appears to be *Entomophthora sphaerosperma*.

Some Pathological Problems in Connection with the Development of the Plains Shelter-belt Project.—ERNEST WRIGHT.

A branch of the Division of Forest Pathology, United States Bureau of Plant Industry, has been established at Lincoln, Nebraska, in cooperation with the United States Forest Service and Plains Shelter-belt Project. Confronting problems may be of general interest. The numerous species grown, diversity of soil types, and climatic factors, render nursery problems particularly interesting. Root rot and damping off of young deciduous seedlings have been surprisingly common, American and Chinese elm being most universally infected. Control through seed or soil treatments appears necessary for these species. Leaf blights of older nursery stock may require spraying for control in some cases and wilts will be controlled through frequent elimination. Seedling storage problems also are important, especially the Chinese elm root rot caused by *Chalaropsis thielavioides*. Diseases have not yet caused serious losses in recently planted shelter belts. Pressing problems have been to map, before planting, the areas within the southern end of the shelter-belt zone that are infected with Texas root rot. An attempt may be made to locate in advance those areas where noninfectious chlorosis is common. Nematode damage also may prove common in certain areas. With the aid of advanced surveys it is hoped many undesirable areas may be avoided or resistant hosts employed.

Downy Mildew of Hops. CECIL E. YARWOOD.

Fall and spring applications of granular calcium cyanamid and of copper sulphate-lime dust to dormant hop hills caused no significant reduction in the number of systemically infected shoots (spikes) arising from these hills in 1935. Weekly counts of the

number of spikes appearing in a total of 1,860 hills during the spring months indicated that most spike infections were of secondary origin. Under field conditions with dry days and humid nights, there was a regular diurnal cycle of the causative organism *Pseudoperonospora humuli*. Sporangiophores and sporangia were formed during the night, the sporangia were matured in the early morning after dawn and liberated during the day. The alternation of light and darkness is necessary for the expression of this diurnal cycle, and sporangia were not formed when mildewed leaves were subjected to light and high humidity at night. Field and laboratory applications of fungicides were of value in the following order: 0.5% Bordeaux, 1% zinc lime, 1% rosin soap, 1% Palustrex (containing 0.2% copper resinate), 0.25% Coposil, and 0.25% cuprocide. Laboratory studies indicate that differences in the spreading qualities of spray materials cause differences in the amount of spray deposit on the leaves and influence the fungicidal efficiency of these materials.

A Pea Streak Caused by Alfalfa Mosaic.—W. J. ZAUMEYER AND B. L. WADE.

An alfalfa mosaic virus collected at Rosslyn, Virginia, was shown by cross-inoculation studies to be infectious to pea, *Pisum sativum*, producing a decided streaking of the stems, petioles, and leaves. The disease is readily transmitted by the usual mechanical means of rubbing the virus extract on to healthy leaves. Infection was noted 13–15 days after inoculation. The first symptom to appear is a slight purpling and streaking of the stems, a recurving of the stipules, and downward curling and distortion of the leaflets. The streaking may later extend from the base to the tip of the plant. The veins and veinlets of the leaves are slightly cleared at first but later become reddish brown. The apical leaves often manifest a rosetted condition. Infected plants show wilting and often die. The phloem tissue becomes discolored and necrotic. Pods formed before the plants become seriously infected, take on a dark purplish gray discoloration. They may be spotted and pitted and decidedly malformed, and frequently do not reach maturity. of 38 varieties of peas tested, no resistance was shown by any. The disease has not been reported wide-spread under field conditions. It apparently is not identical with the pea streak described by Linford in Hawaii.

Pea Mosaic and its Relationship to Other Legume Viruses.—W. J. ZAUMEYER AND B. L. WADE.

Field surveys showed pea mosaic usually more severe along borders of fields than elsewhere, suggesting the possibility of the transmission of certain legume viruses to pea. Cross-inoculation studies showed that the mosaic viruses of red clover, white clover, white sweetclover, and alsike clover are infectious to pea. It is probable that other mosaic viruses may be found in the several legumes that may react differently from those described, in spite of symptom similarity. Forty-two varieties of peas were tested for resistance and susceptibility with the several viruses. Of a total of 3057 seeds collected from mosaic-infected plants, 11 produced diseased seedlings. All infected seedlings were produced from seed collected from above the point of noticeable infection. In addition to the production of symptomatological differences on certain pea varieties, the several viruses can be separated on the bases of the reaction of Stringless Green Refugee, Corbett Refugee, Robust, and Resistant Great Northern beans, and of *Vicia faba*. It is believed that mosaic-infected legumes play an important part and are responsible for much of the disease present in the pea crop. Their importance in relation to the spread of the several viruses to pea is that they offer a continuous source of infection.

THE ANTHRACNOSE OF CURRANT AND GOOSEBERRY CAUSED BY PSEUDOPEZIZA RIBIS[1]

E. C. BLODGETT[2]

(Accepted for publication February 9, 1935)

Anthracnose of currant and gooseberry caused by *Pseudopeziza ribis* Kleb. has been the subject of numerous investigations in Europe and America for over 45 years. Frequent notes on the occurrence and severity of the disease have appeared and numerous efforts have been made to develop control measures. However, the disease has received little critical attention since the valuable work of Klebahn (18) in 1906. The primary objects of the present paper are to provide a survey of the literature, to record certain studies on the physiology and life history of the fungus, and to evaluate certain factors important in the epidemiology and control of the disease.

THE DISEASE

The occurrence of the disease in Europe on *Ribes rubrum* L. was reported in 1867 (17), and 6 years later it was first reported in America by Berkeley (1) on the cultivated black currant, *Ribes nigrum* L. Although the disease was regarded by Dudley (8) in 1889 as becoming important in the United States, it apparently attracted little critical attention either in America or in Europe until about 1899, when it became serious. Klebahn (18) discovered the ascigerous stage and named the fungus *Pseudopeziza ribis* in 1906. Although the disease was reported by Carruthers (4) in England as early as 1903 and undoubtedly was present before, it did not become of great importance there until about 1924, as pointed out by Briton-Jones (2).

The distribution of anthracnose is almost world-wide with the cultivation of the hosts. It has been reported from most of the countries of Europe from Norway to Italy, from Asia, New Zealand, Australia, every Province in Canada, from Alaska, Mexico, and the following States of this country: California, Colorado, Connecticut, Delaware, Idaho, Illinois, Indiana, Iowa, Massachusetts, Michigan, Minnesota, Missouri, Montana, New Hampshire, New Jersey, New York, North Dakota, Ohio, Oregon, Pennsylvania, South Dakota, Vermont, Virginia, Washington, and Wisconsin.

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station.

[2] The writer wishes to express his special appreciation to Dr. G. W. Keitt, under whose direction this study was made, for helpful advice and kindly criticism throughout. He is also grateful to Dr. E. M. Gilbert for help in histological studies and to Dr. J. J. Davis for identifying the fungus. Thanks are due to Mr. Eugene Herrling for the photographic work.

The importance of the disease has been largely underestimated because of the nature of the injury. Direct losses may reach 75 per cent of the crop through fruit infection, defoliation, and subsequent sunscald of the fruit. Furthermore, the plants may be so reduced in vitality, and hence rendered more susceptible, that successive crops may be reduced by 50 to 80 per cent. The evidence presented by Ewert (11) and Müller (27), and confirmed during the present study, although no data are included here, show that fruit from bushes severely attacked by the fungus is low in sugar and very poor in quality. Anthracnose is considered the most widespread and destructive disease of cultivated currant and gooseberry. While not of direct economic importance on wild *Ribes* spp., it is a very serious pest on these plants.

The suscepts of *Pseudopeziza ribis* include approximately 25 species of Ribes plants of diverse nature and wide distribution. The list includes all common species of both currant and gooseberry and many wild ones, but no host outside the genus Ribes has been reported. One of the limiting factors in either a study of or report on susceptibility of both species and varieties is the extremely confused state of nomenclature of Ribes plants. This fact has prevented the writer from including here a list of reported suscepts because it would only add to the confusion.

The symptoms of anthracnose on Ribes vary under different conditions and more especially on different hosts. The fungus may attack all except woody portions of the plant above ground, but injury is most common on the leaves (Plate I, A, B, F). Here the manifestations of the disease first appear as small discolored areas that later enlarge and often coalesce, forming large brown patches visible on either surface. Depending on the species or variety affected, the type of lesion varies greatly; but, characteristically, it is dark brown, one to several mm. in diameter, circular or angular, and sharply delimited, though often with a light yellow border. With the first appearance of the disease, or shortly after, the leaves may turn yellow, quite commonly leaving a green zone around individual lesions. On certain wild species of Ribes and some cultivated gooseberries, the spotting is often accompanied by brilliant reddish or purplish pigmentation of the surrounding tissue. Typically the fungus attacks the older foliage first and especially that of bushes in poor vigor or of varieties naturally weak in growth. Premature defoliation, which may be complete by the middle of July, always is associated with anthracnose. With very severe infection, the leaves may turn brown, wither and fall, as if scorched. There is, however, no indication that the mycelium from a single lesion may infect the whole leaf and finally the entire plant, as reported by Laubert (21). Several acervuli may develop on each old lesion. Here masses of conidia, bound in a water-soluble slime, appear as white tendrils or dull translucent drops (Plate I, D). Generally

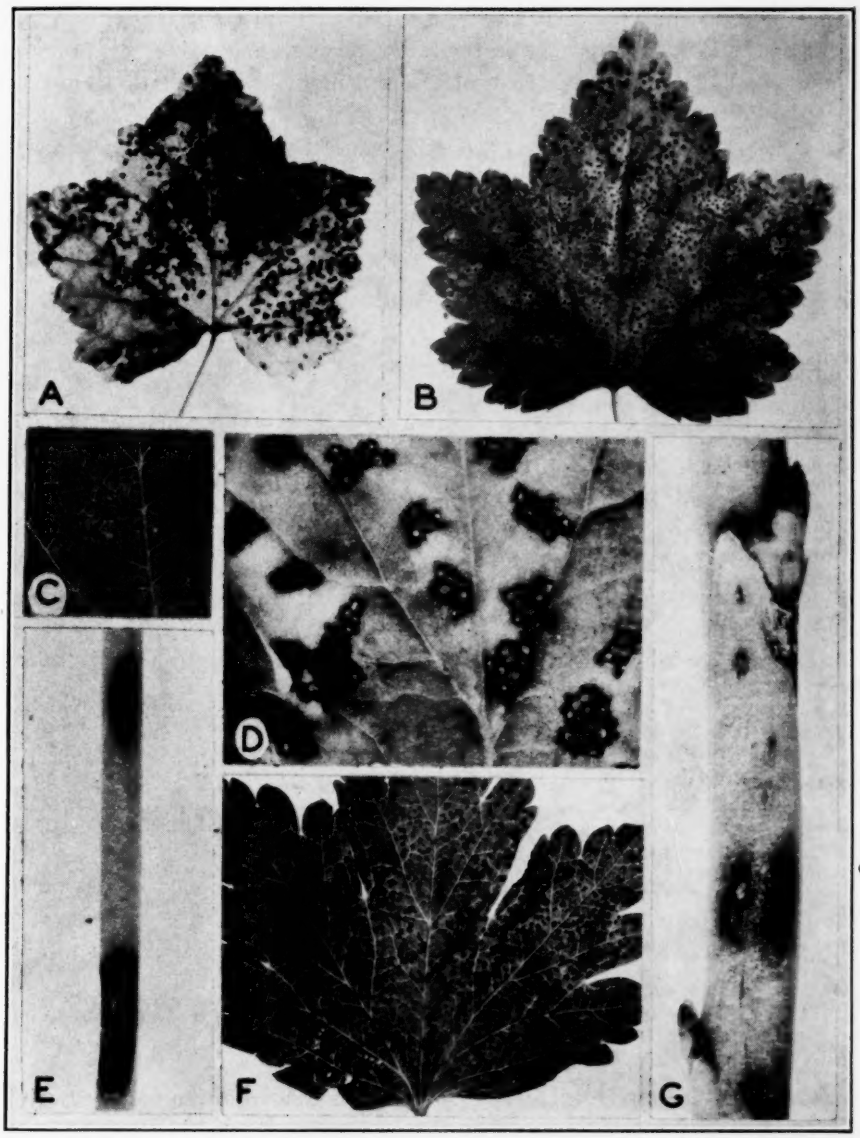

PLATE I. Symptoms induced by inoculations of currant and gooseberry with *Pseudopeziza ribis*. A. Red Cross leaf inoculated with conidia from currant, showing severe type of infection. Slightly reduced. B. Diploma leaf inoculated with ascospores from currant. Slightly reduced. C. Portion of Diploma leaf inoculated with ascospores from currant, using a special technique to limit infection to a certain area. ×1. D. Red Jacket leaf inoculated with conidia from gooseberry, showing lesions produced and abundant sporulation. ×3. E. Red Jacket petiole inoculated with ascospores from gooseberry. ×4. F. Red Jacket leaf inoculated with ascospores from gooseberry. Slightly enlarged. G. Diploma cane inoculated with ascospores from gooseberry. ×3.

these form on the upper surface of the leaf, but in some cases they may occur on both surfaces or only on the lower. Final diagnosis of anthracnose is made by the presence of the typical crescent-shape conidia of the causal organism (Plate II, A–G).

On the petioles and pedicels the lesions may girdle the organ, producing a small canker (Plate I, E). The symptoms on the fruit appear as tiny black specks, but the attack may cause severe shelling of the fruit, as reported by Clinton (5) and shown in his Plate IVa. On the young nonwoody shoots the lesions appear as superficial golden brown areas (Plate I, G).

THE CAUSAL ORGANISM

Eleven isolates of the fungus have been used in this study. Their origin is indicated in the following notes:

1. From conidia on leaves of either Cherry currant or of gooseberry, Sturgeon Bay, Wis., July, 1931.

2. From conidia on cultivated red-currant leaves sent through the courtesy of H. P. Barss, from Corvallis, Oregon, July, 1931.

3. From conidia on cultivated red-currant leaves sent through the courtesy of W. L. Gordon, from Winnipeg, Manitoba, Sept., 1931.

4. From conidia on Poorman leaves, Madison, Wis., Oct., 1931.

5. From conidia on cultivated red-currant leaves, Madison, Wis., Nov., 1931.

6. From conidia on Franco-German leaves, Madison, Wis., Oct., 1932.

7. Culture from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, Jan., 1933.

1 C. From ascospores on overwintered Cherry currant leaves, Sturgeon Bay, Wis., May, 1933.

1 G. From ascospores on overwintered gooseberry leaves, Sturgeon Bay, Wis., May, 1933.

4 G. From ascospores on overwintered gooseberry leaves, Madison, Wis., April, 1933.

6 C. From ascospores on overwintered Franco-German leaves, Madison, Wis., April, 1933.

Some isolations were extremely difficult to make from the material available, because the fungus grows very slowly (especially isolates 3 and 5) and occasionally certain lots of spores, for no determined reason, failed to germinate. These difficulties have been mentioned previously by Ewert (11), Klebahn (18), and Luijk (23). Spore masses, naturally formed or forced out on leaves in a moist chamber, were removed with a sterile needle to nutrient agar and, later, pure culture transfers were made. Ascospores were allowed to germinate after being discharged (on nutrient agar) from

portions of leaf bearing ascocarps. Single-spore cultures were secured from isolates 1 to 4, inclusive, but only after many failures in the case of No. 3. Numerous attempts were made with spores of isolate 5 but without success. All of the studies reported in the case of isolates 1–4 refer to the single-spore cultures. Stock cultures were maintained on potato-dextrose agar kept in the ice-box or at room temperature.

The identification of the fungus as *Pseudopeziza ribis* from the leaf specimens mentioned above was done by the writer and confirmed by Dr. J. J. Davis.

The morphology of *Pseudopeziza ribis* cannot be treated adequately within the scope of this paper, so mention is made only of pertinent facts, and the reader is referred to Laubert (21), Klebahn (18), Janczewski and Namyslowski (14), and Luijk (23). An examination of the conidia produced by the different isolates in culture showed consistent variation in size and shape of the spores. In view of the usual disagreement of workers on the size and shape of conidia of *Pseudopeziza ribis* it seemed advisable to determine the question for the isolates concerned in this study. One hundred spores from the original leaf material for each isolate were measured as usual, for width taking the maximum diameter and for length taking the shortest distance between the most extreme parts of the spore regardless of curvature. Then, for each isolate, 400 spores taken from colonies grown on potato-dextrose agar at 20–24° C.[3] were measured as above. Since these values did not compensate for the curvature of the spore, a suspension of representative conidia from culture was placed on a clear agar medium and photographed. The photograph was then projected onto paper and the outline of the spores drawn. Using a ruler and a rather stiff copper wire, 2 measurements were taken: first, the same measurement as above and, second, the length of the line from end to end equidistant from the sides of the spore. By proportion the corrected or actual length of the conidia in culture was computed. The results of these measurements and comparisons are shown in table 1 and by the photographs in plate II, A–G. The data indicate that the spores in culture are much larger than those produced on the host.

To determine the effect of different hosts on the spore size of one strain, the following experiment was performed. A Red Jacket plant was inoculated in February, 1934, with ascospores from gooseberry leaves from Sturgeon Bay, 1933. When conidia were available on this plant they were used in inoculating 3 varieties of currants and 2 of gooseberries and all bushes were kept under the same conditions. After the lesions were well formed the leaves were placed in a moist chamber and after sporulation, 50 conidia were measured as usual. The results showed only slight differences in size

[3] Temperature is expressed throughout this paper in degrees Centigrade.

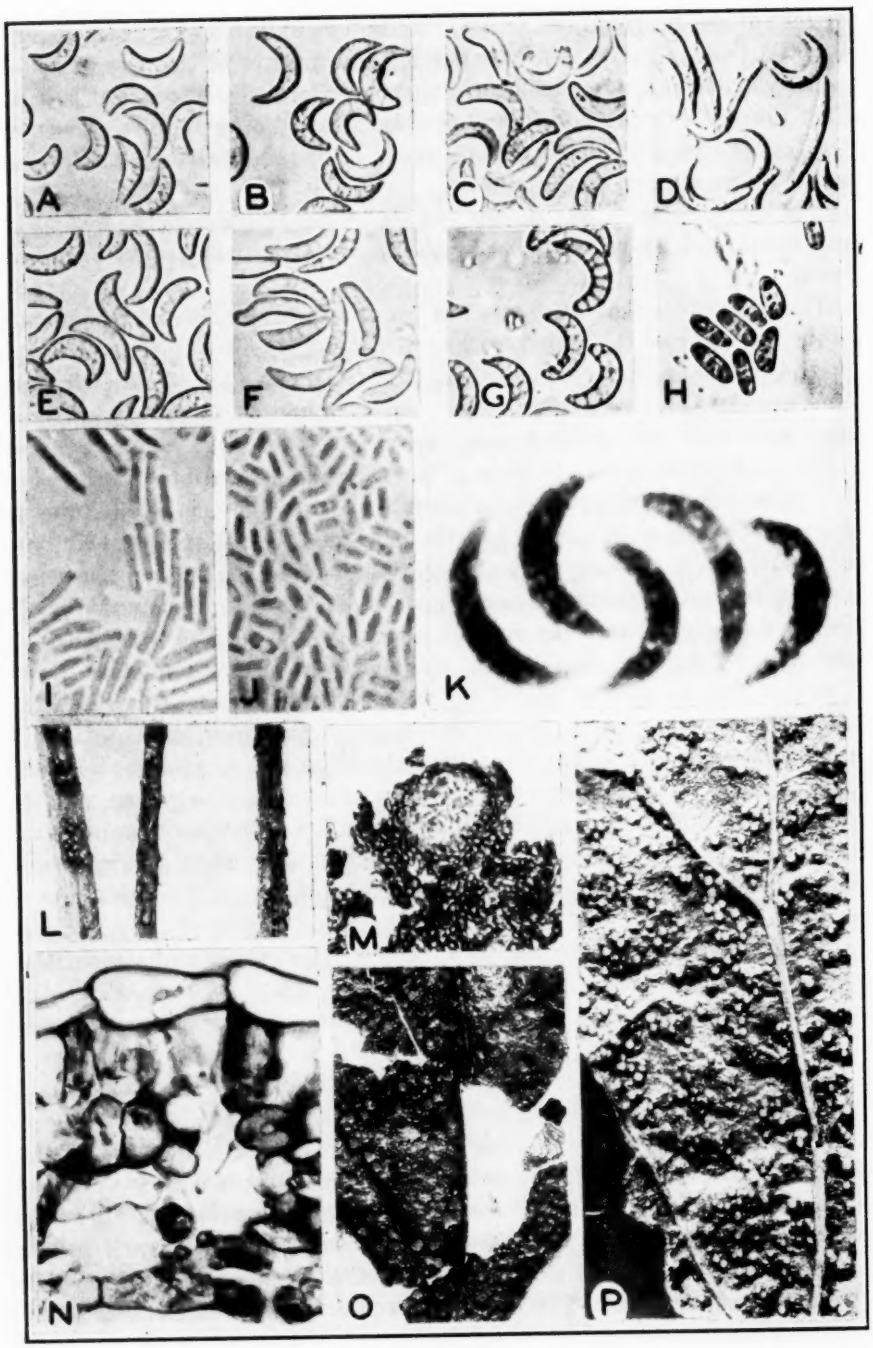

PLATE II

and spore shape. The range was 20.5 to 23.0 microns in average length on Diploma and Cherry, respectively, and 5.3 to 5.9 in average width on Wilder and Downing, respectively. Conidial fruiting was sparse in some cases, however, sometimes accompanied by the production of microconidia.

The effect of temperature on size of spores was determined by measuring 30 conidia in each of 2 tests with leaf material incubated at temperatures of 4–32° at 4° intervals. As a check on these results, 50 spores at 4, 12, 16, 20, and 28° and 100 spores at 8 and 24° produced by isolate 3 growing on potato-dextrose agar for 12 weeks were measured as usual. These results indicated that there was little difference in size and shape of spores produced at the different temperatures. The range in average length was from 19.8 μ at both 20° and 32° to 22.5 μ at 22°; and in average width from 5.0 μ at 20° to 6.0 μ at 4°, 8°, 22°, 28°. Conidia in culture, however, were larger than those found on the leaves and varied in average length from 25.2 μ at 28° to 29.7 μ at 24°; and in average width from 5.8 μ at 28° to 6.7 μ at 16°.

Conidia have granular or extremely vacuolate contents and, although very few spores in nature possess a cross wall (until germination), such walls are common in culture. Spores fixed in very dilute Flemming's weak fluid and stained with Delafield's haematoxylin show the presence of one nucleus (Plate II, K).

Microconidia in cultures of *Pseudopeziza ribis* were first described by Klebahn (18), but he was very cautious about admitting their connection with the fungus under study. Janczewski and Namyslowski (14), found them in abundance on the host. These spores in nature as in the case of conidia vary greatly in size. They are usually rod-shape with rounded or quite blunt ends. Some appear uniseptate, but the identity of the wall is uncertain. They are granular or vacuolate, hyaline, and appear to be uninucleate. Microconidia from isolate 2 grown on green bean pods in test tubes were 8.9 × 2.3 μ in size, as shown by an average of 400 measurements. A comparison of microconidia from the 7 isolates grown on potato-dextrose agar at 20° for 8 weeks shows 2 distinct classes. Isolate 2 forms large microconidia 11.2 × 1.6 μ (Plate II, I) and the other 6 isolates small ones,

PLATE II. A–G. Unstained conidia of isolates 1–7, respectively, from potato-dextrose agar. ×350. H. Unstained ascospores from gooseberry leaves. ×335. I and J. Unstained microconidia of isolates 2 and 4, respectively, from potato-dextrose agar. ×850. K. Conidia of isolate 1 stained with Delafield's haematoxylin, showing uninucleate condition. ×1180. L. Ascocarps on overwintered Franco-German petioles. ×3. M. Overwintered Franco-German leaf, showing conidia borne in a pycnidium-like body. ×165. N. Downing gooseberry leaf, showing the primary hypha after penetration by the fungus. Further development has occurred involving the entire area shown, with many cells completely filled with mycelium and some already killed. ×500. O. Fragment of overwintered gooseberry leaf covered with mature ascocarps. ×3. P. Fragment of Franco-German leaf covered with mature ascocarps. ×3.

TABLE 1.—*Size of conidia of the different isolates of Pseudopeziza ribis produced respectively on the leaf and in culture*

Source of spores	Measurements in microns from stated isolates:[a]						
	1	2	3	4	5	6	7
Gooseberry leaf	22.6 × 5.3	20.5 × 5.7	.....	24.9 × 5.5	.....	.....	.....
Currant leaf	18.9 × 5.4	.....	17.1 × 5.8	.....	18.0 × 5.3[b]	18.0 × 5.7	.....
Spores from colonies on potato dextrose agar at 20–24°	21.6 × 6.7	23.1 × 7.0	27.1 × 7.0	25.8 × 6.4	25.7 × 7.2	27.4 × 7.3	27.3 × 7.1
Do. (Actual length of spores)[a]	28.1	33.9	33.9	32.8	32.0	30.2	36.8

[a] See text for methods used and number of measurements.

[b] These leaves were collected in the same isolated garden the following year. In all other cases the leaf material represents the source of the original isolate. See notes earlier in the paper.

those from isolate 4 (Plate II, J) measuring 4.8×1.4 μ, each measurement being an average from 100 spores. The rôle of these small bodies in the life history of the fungus is unknown, but it is probably in connection with fertilization, as demonstrated recently in other fungi by Dodge (6), Drayton (7) and others.

Klebahn (18, 19) has reported the perfect stage of *Pseudopeziza ribis* on *Ribes rubrum* and *R. nigrum,* but considers (20) that the fungus on *R. grossularia* has lost its power of producing apothecia. The writer has found ascocarps of the fungus on *R. rubrum,* on the wild black currant (*R. americanum* Mill.) and on *R. grossularia.* Rather wide variations occur in the size and shape of the ascocarps on both currant and gooseberry, but no characteristics were consistent enough to distinguish the fruiting bodies on the different species (Plate III, H, I). The average measurements of 500 ascospores from each *R. rubrum* (Franco-German) and *R. grossularia* (cultivated) were, respectively, 15.5×6.7 and 20.2×9.3 μ. The ascospores on currant were matured at 12°–16° during April, 1933, at Madison and after being discharged on 1.7 per cent agar in water were preserved in 70 per cent alcohol. Those on gooseberry matured in nature at Sturgeon Bay and were measured after being discharged on 1.7 per cent agar in water.

PHYSIOLOGICAL STUDIES

Sporulation. Conidia may be present on leaves at the first macroscopic evidence of the disease and continue to form as long as the leaves remain on the bush. A moist atmosphere greatly accelerates spore production. The effect of temperature on sporulation of the fungus on gooseberry leaves was determined by 3 experiments. Leaves of Red Jacket bushes, artificially inoculated in March, 1933, and kept in the greenhouse, were picked on April 27 and, after being washed to remove nearly all the spores, were placed in moist Petri dishes and held at temperatures from 4 to 32° at 4° intervals. After 48 hours, records were taken by rating the leaves in regard to abundance and size of spore masses (Plate I, D). This experiment was repeated in February, 1934, using Downing leaves with smaller lesions and extending the temperature range from about 1° to 36°. Another test in March, 1934, was conducted similarly, using Downing leaves that were thoroughly brushed and washed to remove essentially every spore. In the last test on leaves below 8° and above 28° very few spores were formed; indeed, at more favorable temperatures they were sparingly produced. It is considered that injury to the acervuli in brushing accounts for the difference from the results of the earlier experiments. As a result of these tests it is concluded that sporulation will occur at any temperature generally tolerated by the growing host, the optimum falling between 20° and 28°. It also was found that spores produced at temperatures from 4° to 32° would germinate on

agar at 16°. Only abnormal spores were formed at 36° and none of these tested would germinate. Field observations and tests indicate that viable spores are present even during extremely hot dry periods of the summer.

In pure culture the fungus usually produced conidia abundantly, the several isolates, however, behaving very differently. White glistening drop-like masses composed of spores were formed in all isolates except No. 7. Age of the culture at which sporulation occurred varied widely, isolates 1, 2 and 4 fruiting first and 3 and 5 much later. Of the media used, sterile green bean pods and potato-dextrose agar were most conducive to fruiting of the fungus. The colonies grown on potato-dextrose agar in 6-oz. bottles in the temperature series of 1933 and 1934 were examined and the temperature curve for sporulation secured. The 2 colonies in each bottle were scraped with a wire hook and water suspensions made on glass slides. Three bottles of each isolate at each temperature for the 2 series were examined at the end of 8 weeks. The abundance of conidia and microconidia was rated in values from 1 to 7. These are averaged and shown in table 2. The results indicate that the optimum temperature for conidial production of the fungus

TABLE 2.—*Relation of temperature to the production of conidia and microconidia of Pseudopeziza ribis on potato dextrose agar*[a]

Temperature	Relative abundance of spores produced by stated isolates:							
	1	2	3	4	5	6	7	Av.
Conidia								
4°	.1	.2	.8	0[b]	.7	.2	.1	.3
8	.1	.2	1.4	0	.5	1.0	.2	.5
12	0	.2	2.4	0	.2	.2	.4	.5
16	.2	.2	3.8	.2	3.2	2.2	.7	1.5
20	3.7	1.3	5.8	1.7	7.0	7.0	3.2	4.2
24	5.8	1.7	6.8	7.0	4.7	7.0	4.7	5.4
28	3.5	.8	.8	6.8	.4	1.3	2.5	2.3
32	.....[c]	.....	.....	.....	.....	.....	.....	.....
Microconidia								
4°	2.6	1.9	.3	4.2	.8	.9	.7	1.6
8	5.0	5.7	.3	4.7	1.0	2.3	2.3	3.0
12	6.8	7.0	.4	6.2	1.1	2.3	1.7	3.6
16	6.8	7.0	.8	5.5	1.1	3.0	1.0	3.6
20	6.3	3.3	.3	5.2	.3	.4	1.0	2.4
24	2.1	2.5	.3	3.3	.5	0	1.1	1.4
28	.4	.6	.1	1.3	.3	0	1.1	.5
32	.....	.....	.....	.....	.....	.....	.....	.....

[a] See text for conditions of the experiment.

[b] No spores produced.

[c] No growth of the fungus.

under these conditions is about 20°–24°. Under these same conditions temperatures of 8°–16° were more favorable for the production of microconidia (Table 2).

Similar tests were made on colonies of the fungus growing in 12-oz. bottles at 20°–22° for 8 weeks on a special medium at different pH concentrations. One bottle of each of the 3 series was examined for each isolate at each pH and the records are averaged and shown in table 3. From these data it appears that conidial production was greatest under these conditions at about pH 4 and microconidial production was abundant whenever growth occurred.

TABLE 3.—*Relation of pH of the medium to the production of conidia and microconidia of Pseudopeziza ribis*[a]

Isolate	Relative abundance of spores produced at stated pH:							
	3.02	3.98	4.98	5.40	5.96	6.97	8.12	9 ±
Conidia								
1	0[b]	4.7	.7	1.7	2.0	1.3	1.0	.....
2	0	3.7	3.3	6.3	4.3	2.0	1.3	.....
3	.....[c]	.7	.7	0	0	0	0	.....
4	.....	3.3	3.3	5.3	5.3	6.7	2.3	.....
5	.....	2.0	1.3	1.0	.3	1.0	0	.....
6	.....	6.3	6.7	4.0	2.3	.3	0	.....
7	.....	.5	.5	0	0	0	0	.....
Av.	0	3.2	2.4	2.6	2.0	1.6	.7	.....
Microconidia								
1	0	6.3	7.0	7.0	7.0	7.0	2.3	.....
2	0	5.7	6.7	5.0	5.3	5.0	7.0	.....
3	.....	1.3	2.7	2.3	2.0	2.0	2.7	.....
4	.....	7.0	5.3	5.3	3.0	7.0	6.3	.....
5	.....	2.0	2.7	2.3	4.0	2.0	4.7	.....
6	.....	5.0	1.7	1.0	.7	1.0	1.0	.....
7	.....	2.5	1.7	5.0	5.0	6.7	6.0	.....
Av.	0	4.3	4.0	4.0	3.9	4.4	4.3	.....

[a] See text for conditions of the experiment.

[b] No spores produced.

[c] No growth of the fungus.

Microconidia may be formed in nature at any time during the summer but most abundantly in the latter part of the season. They also may appear in great numbers along with conidia on overwintered leaves bearing ascocarps. Artificially, they are produced more commonly on old cultures or under adverse nutrient on environmental conditions.

Ascospores are produced in apothecia borne in the spring on overwintered leaves and petioles (Plate II, L, O, P). No ascospores have been seen in artificial cultures, although bodies suggestive of perfect fruiting structures are frequent on the mycelium formed in a "secondary growth" of old cultures. This rapidly growing mycelium later becomes dark, with thick walls, and appears much like that formed in leaves during the fall and early winter.

Germination. Ascospores germinate readily in drops of water, on 1.7 per cent agar in water, and on potato-dextrose agar. The germ tube apparently may arise at any place on the surface of the spore, producing in some cases a rather large appressorium and a thick stocky germ tube that generally branches profusely and forms a dense mass of septate hyphae. Germination may begin in a few hours but proceeds slowly. Klebahn (18) figures ascospores within the ascus germinating and producing conidia.

Reports on the germination of conidia have resulted in contradictory evidence. Ewert (11) reported after numerous trials that spores would germinate only after the fall frosts. Luijk (23) found that spores from *Ribes nigrum* would not germinate in July or after storage for a month in the laboratory. In August the form of *R. grossularia* germinated readily. The writer has found that spores, both in nature and in pure culture, may germinate, or they may not, any time after they are formed. These facts have presented great difficulties some of which have been overcome, but the reasons for such behavior have not been formulated. It will thus be seen that the selection of spores for germination tests has been at best somewhat unsatisfactory. However, all precautions possible were taken to use only comparable material for tests reported herein. In most cases spores were taken from colonies on potato-dextrose agar or on bean pods by removing the spore horns with a wire hook and making a suspension in sterile distilled water. Preliminary trials indicate that spores may germinate in all concentrations of suspensions from a few per low-power field to many hundreds, the advantage, in general, being with the medium to heavy concentrations. The germ tube may arise anywhere on the surface of the spore, though generally near the end. Frequently, during germination, the spore becomes uni- or multiseptate. Spores of isolate 5 produce relatively thick, compact germ tubes, while those of isolate 4 send out thin, thread-like germ tubes. Appressoria may be produced.

Temperature Relations. The effects of temperature and of 2 common media on the germination of conidia at different time intervals are shown in table 4. Although these data were secured by a single test in duplicate involving generally 100 or more spores, they are representative of results from several series of similar experiments using spores from the same and other sources. Conidia were placed in sterile, double-distilled water, and

TABLE 4.—*Relation of temperature and of two different media to the germination of spores of Pseudopeziza ribis*

Isolate	Spores and medium[a]	Hours	Per cent germination at stated temperatures:							
			4°	8°	12°	16°	20°	24°	28°	32°
	Conidia									
1	Double	24	0	0	0	8.9	21.5	4.2	0	0
	distilled	48	0	2.8	52.6	42.9	34.8	13.0	0	0
	water	96	0	22.2	.....	57.9	43.3	21.8	0	0
		24	1.4	7.2	7.0	23.3	10.0	12.1	8.3	0
2	Do.	48	7.4	22.8	15.7	24.8	18.9	26.3	8.3	0
		96	36.5	38.4	31.0	26.6	35.5	28.2	9.0	0
		24	0	0	0	0	0	0	0	0
3	Do.	48	0	0	0	0	0	0	0	0
		96	0	0	0	0	2.9	0	0	0
		24	4.7	6.0	14.3	40.3	42.3	39.6	2.9	0
4	Do.	48	18.7	27.3	47.6	43.0	42.4	50.0	16.9	0
		96	19.6	44.8	66.0	79.2	65.7	57.3	41.3	0
		24	0	0	0	0	0	0	0	0
5	Do.	48	0	0	0	2.1	0	0	0	0
		96	0	0	0	4.0	0	0	0	0
	Conidia	24	9.6	13.6	48.7	69.1	72.4	54.4	19.8	0
1	1.7% agar	48	39.5	38.8	76.3	83.8	89.0	86.2	27.4	0
	in water	96	78.3	77.8	80.3	87.5	93.2	.....	31.3	0
		24	15.4	21.8	48.5	78.8	79.4	82.6	64.1	0
2	Do.	48	50.7	52.2	71.2	82.9	95.4	90.8	74.6	0
		96	76.2	71.2	81.3	88.2	.....	.....	.....	0
		24	0	0	0	0	0	0	0	0
3	Do.	48	2.1	11.5	5.0	35.0	23.8	0	0	0
		96	55.6	75.0	76.7	88.9	59.6	4.8	0	0
		24	27.0	27.5	44.1	75.7	93.0	92.9	78.9	0
4	Do.	48	52.4	61.7	74.7	86.9	95.1	94.7	91.6	0
		96	84.4	70.8	78.1	.....	.....	.....	.....	0
		24	0	3.2	24.6	35.1	20.5	20.3	0	0
5	Do.	48	48.6	39.7	73.4	68.4	68.2	33.8	0	0
		96	66.7	71.7	88.0	91.1	86.8	55.4	3.2	0
	Ascospores									
	1.7% agar	40	76.0	83.3	81.2	75.5	49.3	36.7	30.2	0
	in water									

[a] Conidia in hanging drops. All conidia from fungus grown on bean pods at 20° for about 30 days. Ascospores from overwintered Franco-German leaves.

hanging drops were made, using chemically cleaned glassware. Drops of this suspension were placed also on 1.7 per cent agar in water in Petri dishes and then all spores were incubated at the different temperatures. Besides the readings on germination, the lengths of the germ tubes were measured after 48 hours at the various temperatures and are as follows for spores at 20°:

Isolate	In double-distilled water	On 1.7% agar in water
1	84 microns	210 microns
2	56 do	210 do
3	0 do	14 do
4	126 do	266 do
5	0 do	42 do

The differences shown by the several isolates in both germination and early growth are very great and are typical of their behavior throughout the period of investigation.

To determine the effect of temperature on discharge of ascospores, the following technique was employed for the tests in 1933. Two pieces of currant leaf bearing mature ascocarps were moistened on the under side of a Petri dish lid so that the spores would shoot down onto a film of 1.7 per cent agar in water. The areas were marked and the plates let stand at room temperature for about a half hour to see that spores were discharged in all cases. Then 2 plates were placed in each incubator at temperatures of 4°–32° at 4° intervals and 2 plates were set on ice. All were left for 30–45 minutes and then the lids of the dishes were moved over a new area marked 2. After 48 hours an examination showed that spores had been discharged at all temperatures but most abundantly at 16°–24°. This experiment was repeated, using another technique. The leaf material was dried down at room temperature, and, after it was placed in the incubators for 45 minutes, a new area was marked and the leaf tissue moistened with water from a medicine dropper that had come to the temperature of the incubator. The results confirmed those of the first series.

A similar procedure was used in securing ascospores for germination tests at different temperatures. Spores were discharged in Petri dishes on 1.7 per cent agar in water over a period of about 1 to 1½ hours at room temperature and then the spores were incubated at 4°–32° and on ice. The results of 2 such series are averaged and shown at the bottom of table 4.

Growth of the fungus at different temperatures was compared in an extensive trial in 1933 and repeated in 1934. Twenty-five-cc. portions of potato-dextrose agar were placed in 6-oz. glass bottles stoppered with cotton. After the agar had solidified, while the bottles lay flat, two 4-mm. discs of agar bearing the fungus were planted in suitable position in each bottle. This inoculum was secured by pouring a spore suspension onto potato-dextrose agar and incubating until a mat was formed. A stiff wire loop was used to cut out the pieces. In each of the 2 series, 5 bottles of each isolate were incubated at each temperature and records taken weekly. The data for each isolate are averaged for the 2 tests and shown in figure 1.

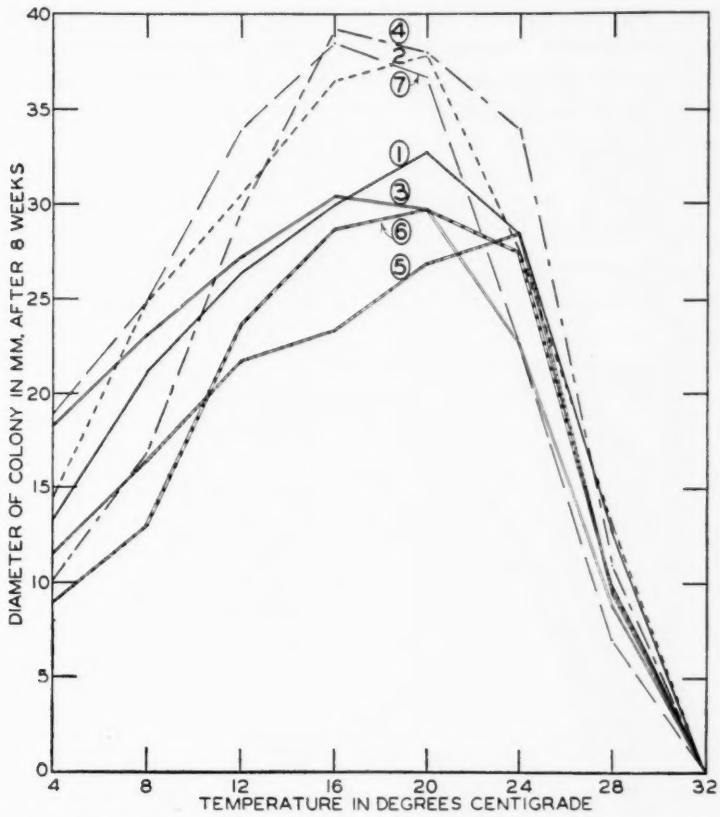

FIG. 1. Effect of temperature on the growth of 7 isolates of *Pseudopeziza ribis* on potato-dextrose agar.

Hydrogen-ion Relations. The effect of hydrogen-ion concentration on the germination of conidia was determined by a series of 4 trials. The medium was prepared as follows: 4 gr. KH_2PO_4, 0.5 gr. $MgSO_4$, 17 gr. agar and 1000 cc. distilled water were mixed and sterilized in 100 cc. amounts. On the basis of extensive preliminary trials certain amounts of sterilized normal NaOH and HCl solutions were added aseptically to each 100 cc. and, after mixing thoroughly, the medium was poured into Petri dishes. Tests on pH were made of representative plates of agar, using the quinhydrone apparatus. The readings of pH were quite consistent in the different series and are averaged for the respective values. Spore suspensions were placed in drops on the agar plates and then incubated at 16° for 72 hours, when the readings were taken. The results are averaged and shown in figure 2. Further comparisons were made, although not presented here, recording the approximate germ-tube length. The curves representing these data closely

approximated those showing germination (Fig. 2), as far as optimum and range were concerned. There was, however, only slight development at pH 8.42, whereas the spores germinated fairly well. Isolate 7 made the best growth and isolate 3 the poorest.

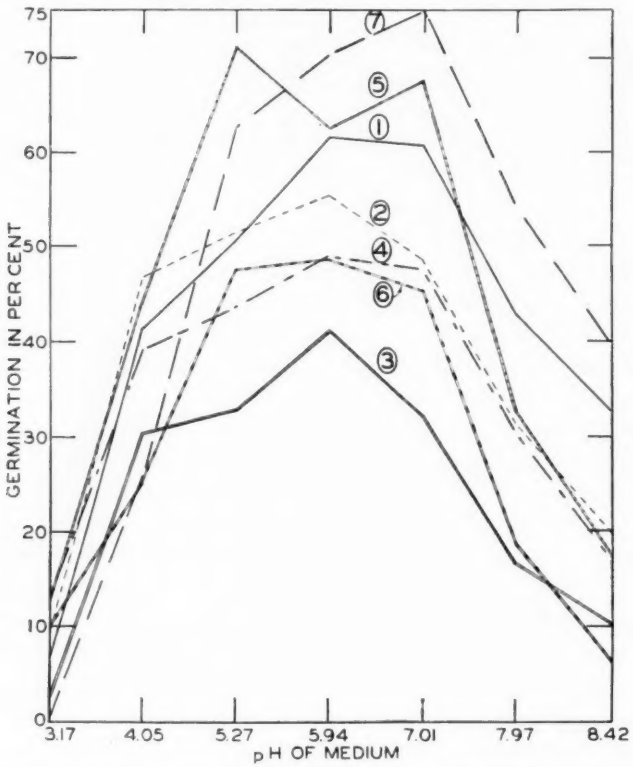

FIG. 2. Effect of hydrogen-ion concentration on the germination of conidia of 7 isolates of *Pseudopeziza ribis* on a special medium after 72 hours at 16°.

Growth of the fungus on a medium in which the pH varied was determined in 3 sets of experiments in 1933. The medium consisted of potato-dextrose agar to which were added, 4 gr. KH_2PO_4, 0.5 gr. $MgSO_4$ and 10 gr. peptone per liter. Fifty cc. portions of the medium were sterilized in 12-oz. cotton-stoppered bottles. Just before the agar was ready to set, certain amounts of sterilized normal NaOH and HCl solutions were added aseptically. Some bottles were left without adding acid or alkali (pH 5.4). After being thoroughly mixed, the medium was cooled and set with the bottle lying flat. Using similar fungus mats as for the temperature series, 2 discs, 4 mm. in size, were placed in suitable position on the agar surface and the bottles incubated at 20°–22°. Readings were taken each week. One

bottle at each pH was used for each strain in the first test and 2 bottles each in the other 2 tests. All the results are averaged and shown in figure 3. This medium proved to be very well adapted to growth of the fungus. Changes in pH, which were determined for the media after the test, show the following general results. At pH 3 only very slight change in the medium, in proportion to the amount of fungal growth, occurred for the isolates tested (2–5, inclusive). At pH of 4, 5, 5.4, 6, 7, and 8 the medium changed toward approximately pH 7.5, the amount of change being directly correlated with the extent of fungus growth. At approximately pH 9, no growth of the fungus occurred, while the medium adjusted itself to about pH 8.6.

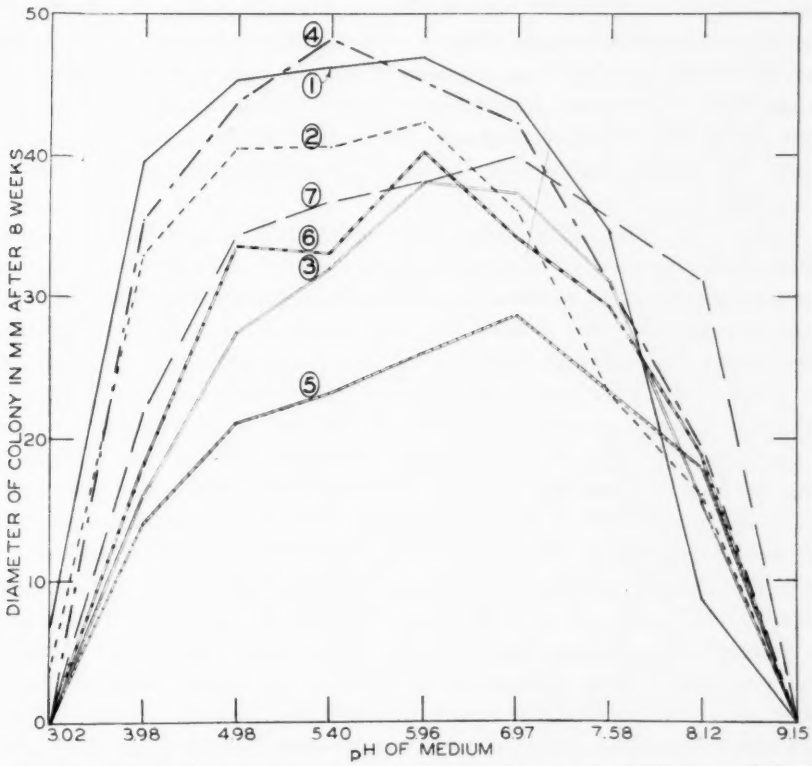

FIG. 3. Effect of hydrogen-ion concentration on the growth of 7 isolates of *Pseudopeziza ribis* on a special medium at 20–22°.

The hydrogen-ion concentration of Ribes leaves of several varieties kept under different temperature conditions, was tested with the quinhydrone apparatus. The results showed readings of pH 4.9 to 6.6 for gooseberry and of 5.8 to 7.1 for currant. This range includes values for samples of old and of young leaves taken separately for plants kept at about 28, 19, and 12°.

There appeared to be no consistent correlation of pH with temperature conditions of the host over a period of 2 months (determinations were made February 20, March 20, and April 17).

INFECTION STUDIES

Previous infection studies with *Pseudopeziza ribis* have been very limited. Ewert (11) by spray treatments at short intervals determined in general the time of primary infection in the spring. He also produced the disease by artificial inoculation with conidia. Klebahn (18), while demonstrating the relation of *Gloeosporium ribis* to the perfect stage, secured infection with conidia and ascospores. He was concerned primarily in later tests (19) with cross inoculations, using conidia on various species to demonstrate specialization of the fungus. The studies conducted by the writer indicate that the condition of the host plays so important a part in successful inoculations that conclusions concerning variability of the fungus, or susceptibility of the different species or varieties of the host, have sometimes been in error. At other times, correct, but apparently conflicting, conclusions have been drawn.

Lists of currant and gooseberry varieties with regard to their susceptibility have frequently been published that show conclusively that no definite statements can be made at present on the comparative or relative resistance of varieties. The Wilder has been reported by Stewart and Eustace (29) as very resistant and by Clinton as very susceptible (5). Similar cases are noted with other varieties, but, because of the confusion in varietal names of currants, some such reports are not authentic. The Long Bunch Holland variety (Thayer (32)) which probably includes the Franco-German, the Red Holland and the Red Dutch, illustrates this condition. The Franco-German is one of the most resistant sorts known, but may be heavily infected during late September, October, and November. In Germany (10) the Red Holland is reported as the most resistant variety, but it, too, may be infected late in the season. The Red Dutch is classed as very susceptible by Dutton (9). Briton-Jones (2), in 1925, made some very interesting comments concerning varietal susceptibility. He states that, at first, it would seem that the Baldwin bushes are more susceptible in a genetical sense than other varieties. Such is not the case, since heavily pruned Baldwins were no more affected than heavily pruned bushes of other sorts. The reason why it is more often and more severely affected is due to the fact that it exhausts itself by heavy cropping and is a weak grower. "The heriditary factor, therefore, is not one of susceptibility to disease but one of heavy cropping and weak growth. Without a critical study of the conditions of plants it is unsafe to state that any one variety is more susceptible to disease than another." From such reports in literature and from studies and

observations by the writer, it is concluded that all commonly cultivated varieties of red, white, and black currants, and gooseberries may be infected by *Pseudopeziza ribis*, their comparative susceptibility being influenced largely by environmental conditions.

The infection studies reported in this paper were made on No. 1, 2-year-old currant and gooseberry plants bought from the McKay Nursery Co., Madison, Wisconsin. The bushes were planted in good soil in 8-inch pots and most were kept at about 19°. They were watered sufficiently to prevent severe wilting. By experience it was found that moderate to poor growth of the host was more favorable for infection by the fungus.

In all, over 400 plants were inoculated, using the following varieties: currant, Wilder, Perfection, Cherry, Diploma, White Grape, Fay's Prolific and Red Cross; gooseberry, Red Jacket, Downing and Pearl. All 11 isolates were pathogenic, although in varying degrees, and all varieties tested were susceptible. Conidia of cultures isolated from currant when inoculated on currant gave better infection than on gooseberry. Likewise, isolates from gooseberry were more pathogenic on gooseberry. The plants were inoculated by atomizing the lower, or upper, or both leaf surfaces with a suspension of conidia in distilled water, and also by discharging ascospores onto the leaf surface. The bushes were then placed for definite periods in a moist chamber described by Keitt and Jones (16). Results were taken after 2 weeks or longer.

TABLE 5.—*Relation of temperature of Ribes plants before inoculation to infection of excised leaves by conidia of Pseudopeziza ribis*

Host plant[a]	Temperature before inoculation,[b] ° C.	Results of inoculation of excised leaves on stated dates and leaf surfaces:[d]			
		March 21[c]		April 19	
		Upper	Lower	Upper	Lower
Currant	28	1.52	17.04	2.61	6.45
	12	.75	2.90	1.28	.52
Gooseberry	28	3.12	24.57	80.20	95.00
	19	2.71	12.21	42.70	97.60
	12	.93	6.32	2.87	57.40

[a] These plants are the same used in the experiment on pH of Ribes leaves.

[b] All plants had been growing at about 19° for 30 days when they were placed at these temperatures on February 20.

[c] Excised leaves 19–32 in number were incubated in moist dishes at 20° for two weeks after inoculation with conidia; isolate 5 on currant, isolate 2 on gooseberry.

[d] The average number of lesions on the most heavily infected ¼ Sq. inch per leaf.

The inoculations of excised leaves in moist dishes proved to be as reliable as those on potted plants. It should be pointed out that the behavior of the fungus was as erratic in these studies as it was in spore-germination trials, although no correlation could be drawn between successful inoculations and germination of the spores on agar plates. The data given, while representing rather limited numbers of plants, are considered a fairly accurate representation of the results of the 3 years.

Factors influencing infection. The effect of different temperatures on susceptibility of the plants is shown by the experiments on excised leaves reported in table 5 and by more extensive trials on entire potted plants. With the exception of a single trial on Downing, in tests not reported, the plants held at higher temperatures were much more susceptible. Since records were taken on both old and young leaves, these differences cannot be attributed to the effect of temperature on extent of growth, favored by lower temperatures, but rather to the condition of the plant, particularly the leaf. That morphological changes account for the differences seems unlikely.

In a test on April 25, 1932, leaves of Wilder and an unknown currant were inoculated with spores of isolate 5 after injuring the upper leaf surface. After 2 weeks the numbers of lesions per square inch were as follows:

	Lower surface	Upper surface	Upper surface brushed with a stiff fiber brush
Wilder	11	7	75
Unknown	159	5	200

Other tests and observations have shown that injuries to leaves, or other unfavorable conditions, increase their susceptibility.

The data given in table 6 show conclusively that infection occurs mainly through the lower leaf surface. There are a few exceptions, however, scattered throughout the inoculation experiments. Studies with ascospores show also that penetration is more common on the lower surface.

The length of moist period after inoculation necessary for the fungus to establish itself sufficiently to induce infection is shown to be much shorter than the time curve of germinating spores would suggest. The experiments reported in table 7 permit one to conclude in general that a moist period of 12 to 24 hours is sufficient for infection by conidia. No study has been made of the moist period necessary for infection by ascospores. Extensive data on the relation of temperature during the moist period to infection show that infection may occur at 10° to 28° but none at 32°. In some cases no disease appeared on plants inoculated at 28°. Tests with excised leaves held at 8° gave good infection, but lesions were very slow to appear. The optimum

TABLE 6.—*Comparative susceptibility of upper and lower surfaces of currant and gooseberry leaves to infection by conidia of Pseudopeziza ribis*[a]

Date	Variety	No. of bushes	Surface inoculated	Av. No. lesions per ¼ sq. in. per stated leaf:[b]		
				1	2	3
March 8, 1933	Red Jacket	1	lower	50.0	53.7	30.3
do.	do.	1	upper	4.7	10.0	3.9
March 18	Downing	2	lower	33.9	28.0	13.3
do.	do.	1	upper	16.1	14.0	9.5
March 20	do.	2	lower	11.3	4.7	2.0
do.	do.	do.	upper	1.7	.7	0
March 23	Red Jacket	do.	lower	34.3	31.0	14.1
do.	do.	do.	upper	6.0	3.7	3.1
April 30	Currant	4	lower	37.9	38.6	46.4
do.	do.	3	upper	1.6	.6	.5

[a] The gooseberries were inoculated with spores of isolate 2 from bean pods, the currants with spores of isolate 2 from leaves and of isolate 5 from potato-dextrose agar. The plants remained for 48–86 hours in a moist chamber at 16–20°.

[b] Data were taken on 25 gooseberry and 4 currant shoots per plant, choosing those most heavily infected and counting the lesions on 3 leaves per shoot. These represented the most severely infected leaves on (1) the lower, (2) middle, and (3) upper third of each shoot.

temperature for infection by conidia apparently is from 16° to 24°, as indicated by tests both on potted plants and excised leaves. Ascospores were discharged onto plants held at 12° and at 20° and good infection developed (Plate I, B, F).

Several infection studies were conducted on potted plants and excised leaves using ascospores from currant and gooseberry leaves in 1933 and 1934. In some tests, pieces of leaf bearing ascocarps were moistened on a cover slip placed on a rubber washer. The cell thus made was then clamped on either leaf surface with the ascocarps oriented towards the leaf, and the plant placed in the moist chamber. This method, which was described by Keitt (15), is very useful and the results are striking. Lesions appear in a circular area the size of the aperture of the rubber washer (Plate I, C).

The usual method of inoculating plants with ascospores was to lay the potted plants in the moist chamber at a suitable angle, with the branches so arranged that the lower leaf surface was uppermost and held in place by a tray of large-mesh wire netting. Petri dishes with moist filter paper in the bottom on which adhered Ribes leaves with abundant ascocarps were inverted

TABLE 7.—*Relation of the length of moist period after inoculation with conidia to infection by Pseudopeziza ribis*[a]

Date	Variety	Temperature before inoculation[b] ° C.	No. hours in moist chamber	Av. No. lesions per ¼ sq. in. per stated leaf:[c]		
				1	2	3
April 27, 1933	Diploma	19	12	5.0	6.2	4.2
do.	do.	do.	18	5.0	10.2	12.0
do.	do.	do.	24	6.7	11.6	16.7
do.	do.	do.	48	4.3	4.0	12.9
March 25	Cherry	28	24	2.4	2.0	1.8
do.	do.	do.	48	6.9	9.9	5.7
do.	do.	do.	72	2.7	2.8	1.8
do.	do.	do.	96	2.8	2.9	2.4
April 5	Red Jacket	19	24	33.0	22.2	18.9
do.	do.	do.	48	40.8	36.5	35.0
do.	Downing	28	24	47.1	23.8	9.0
do.	do.	do.	48	95.5	83.4	59.0
do.	do.	do.	72	82.1	84.2	60.2
March 25	do.	do.	48	47.8	46.3	22.1
do.	do.	do.	72	35.1	21.9	11.2
do.	Red Jacket	do.	48	45.2	30.9	25.1
do.	do.	do.	72	61.1	47.5	37.3

[a] In the March 25 test, conidia of isolate 6 from potato-dextrose agar were used on currant; in all others spores of isolate 2 from leaves. After inoculation of the lower leaf surface, the plants were held in the moist chamber at 16 or 20°.

[b] The plants were grown at this temperature since February 20.

[c] Data were taken on the most heavily infected leaf on (1) the lower, (2) middle, and (3) upper third of each shoot. One plant was used in each case, and the data were averaged for 25 gooseberry and for 3–16 currant shoots.

on this wire tray. The plants were sprayed with distilled water and kept in the chamber as long as desired, 48–96 hours. This method of inoculating with naturally discharged ascospores proved very satisfactory, and severe infections were secured (Plate I, B, F). Cross-inoculations, using ascospores from currant, showed definitely that the fungus was more pathogenic on currant than on gooseberry. Similarly, ascospores from gooseberry caused more infection on leaves of gooseberry than on leaves of currant. Because of variations in inoculum, quantitative readings were not attempted. The conidia produced as a result of these inoculations were extremely pathogenic in comparison with spores from artificial cultures. The varieties used were Diploma, Perfection, and Red Jacket, all with growing shoots. Ascospores as well as conidia gave better infection on the older leaves.

To determine whether ascospores could infect very young leaves, however, shoots of Diploma and Poorman were gathered in the garden on April 25, 1933, and, after tying the cut ends in cheesecloth and absorbent cotton, small bunches were fitted like corks in the tops of pint milk bottles containing water. These were partly inverted in a moist chamber and the shoots inoculated as usual with ascospores from currant. When picked, the buds were just beginning to break, and during the moist period they continued to open. Good infection appeared on the small leaves after about 9 days.

Pathological histology. No studies of the manner in which the fungus gains entrance to the host have been reported. Ewert (13), in testing control measures, found that spraying either of the leaf surfaces alone with Bordeaux mixture did not prevent infection, while no disease developed if both surfaces were covered with spray. It would thus appear that infection may take place on both leaf surfaces. Several experiments by the writer with both ascospores and conidia have shown that such is the case. As mentioned before, the uncertain manner in which spores may germinate and infect the host has presented even greater difficulties in penetration studies than in other phases. Many inoculation tests were performed on potted plants held in a moist chamber and on excised leaves in moist dishes, using a wide range of conditions and of methods for applying spores to the leaves. Pieces of tissue were fixed, imbedded, sectioned, and stained. Many variations were tried in fixatives and stains. In all the currant leaves used, certain cells, especially in older tissue, contain materials that stain very heavily and interfere seriously with this operation and with examination. The best results were secured on Downing leaves previously infected and bearing a few lesions. These leaves were picked, washed in a small amount of water, thus spreading the spores from the acervuli, and placed in moist dishes. The wash water was then atomized onto both leaf surfaces and the leaves were incubated at 20°. Fixations were made in Gilson's fluid after 48, 96, and 144 hours. After embedding, the material was sectioned 7–8 μ thick and stained with safranin and fast green. Certain stages of penetration and development of the fungus in the leaf were drawn with the aid of a camera lucida and are shown in figure 4, A–D.

Observations also were made on material treated as follows: Leaves were inoculated by atomizing a spore suspension on one or both surfaces and incubating in moist dishes at certain temperatures. After certain periods of from 24 to 96 hours, pieces of leaf were cut out and the chlorophyll was removed in a solution containing equal parts of absolute alcohol and glacial acetic acid. Then the material was cleared in a saturated solution of chloral hydrate. The tissue was placed on a glass slide in lacto-phenol, heated slightly, and then stained by warming with acid fuchsin dissolved in lacto-phenol. These made very satisfactory temporary mounts for observing

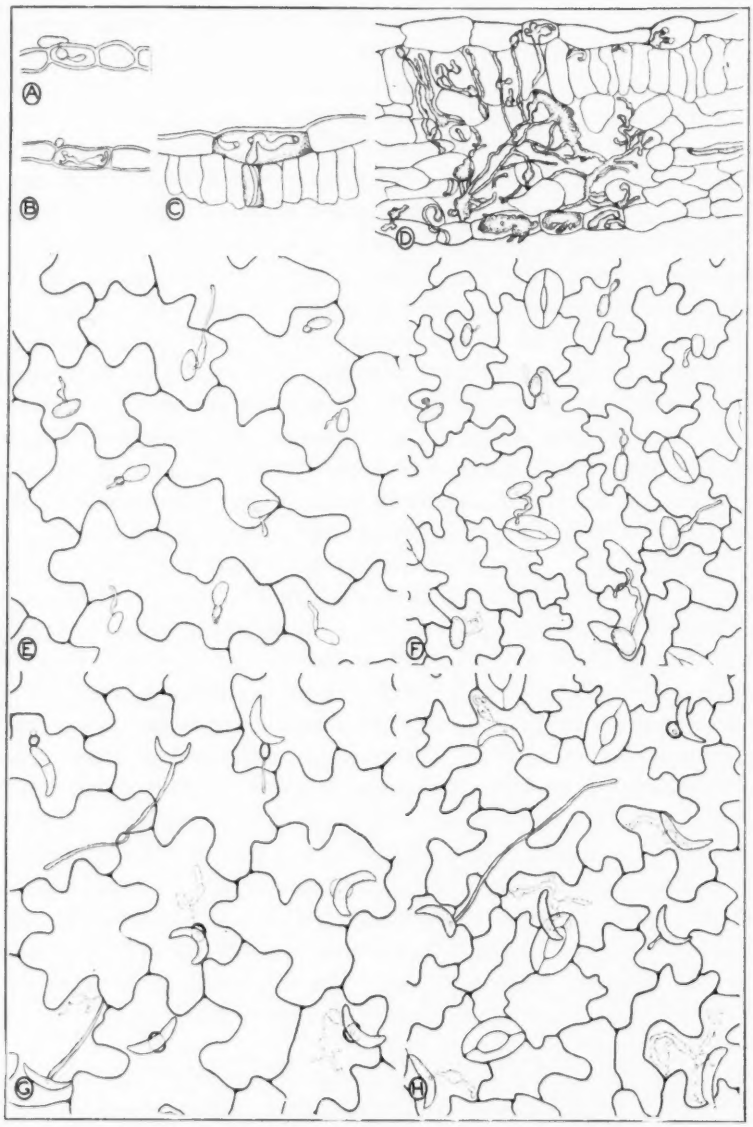

FIG. 4. Penetration and development of *Pseudopeziza ribis* in leaf tissue. Drawings made with the aid of a camera lucida. ×250. A. Early stage of penetration of Downing leaf following germination of conidium. B. Later stage, showing enlarged primary hypha. C. Further development into the palisade layer with killing of the cells. D. Lesion when first visible macroscopically, about 144 hours after inoculation, showing extensive fungus development. E. and F. Ascospores on upper and lower surfaces, respectively, of Diploma leaf. G. and H. Conidia on upper and lower surfaces, respectively, of Downing leaf. Dotted lines represent the fungus inside the leaf.

spore germination and penetration by the fungus. Such studies were made, using conidia on both surfaces of Downing leaves and ascospores on both surfaces of Diploma leaves. Illustrations showing various types of germination and several stages of penetration are given in figure 4, E–H. The mode of penetration in conidial infection by *Pseudopeziza ribis* is shown to be directly through the cuticle and epidermal cell wall.

Conidia that germinate on a leaf surface may or may not produce an appressorium. On the lower surface in many cases the conidium itself acts as an appressorium and the infection hypha penetrates directly beneath the spore. From the spore or from the appressorium, as shown by a germ pore, a small projection or infection hypha is forced down through the cuticle and the epidermal cell wall into the epidermal cell (Fig. 4, H). Here the fungus forms a primary hypha that varies widely in size and shape. Most commonly it is a rather large globular body with one or more branches. These are fine or very large and of irregular shape, generally confined to the same or closely adjacent epidermal cells. This secondary growth may completely fill an epidermal cell (Fig. 4, D) before or simultaneously with the development of slender hyphae that extend into the intercellular spaces and palisade cells, invading all the tissues including the vessels. Large irregular, fungal bodies may be scattered throughout the infected tissue. At about the time the lesions appear the mycelium tends to accumulate just below the epidermal cells, and there fruiting takes place, pushing the epidermis up, rupturing it, and liberating the spores. Parasitized cells die within a short time and may become completely disintegrated, leaving large cavities or spaces filled with fungal growth.

What takes place in the host, if penetration occurs, when a resistant leaf is attacked has not been determined. It is known, however, that spores may germinate readily on leaves that never show lesions. It is possible that in these cases the spores send out long, slender germ tubes that generally stay on the surface. No cases of stomatal penetration were observed. The very simple preparation for penetration, together with the extensive development of the fungus just after entrance, may play an important part in the ready establishment of the fungus. The facts, too, that the cuticle and upper epidermal walls are thicker than those of the lower epidermis may explain in part the data shown in table 6. It should be noted, too, that bushes in proper nutrition possess a thicker cuticle and also more resistance to the disease. Plants grown in the sun, however, are considered to form heavy cuticular layers, but they are also more susceptible than bushes grown in the shade, according to Stewart and Eustace (29).

Studies of penetration by ascospores show essentially the same type as described for conidia (Fig. 4, E, F). Very extensive observation on material from several inoculations, however, showed few examples of penetration;

in fact, on the upper surface very few ascospores were seen germinating and no conclusive evidence of penetration was found. A fair percentage of ascospores germinated on the lower surface, but the fungus was extremely difficult to see after penetration. The writer is of the opinion that the drastic treatment in preparing the leaves literally disrupted the fungal body, leaving granular contents with no definite outline beneath the germinating ascospore.

LIFE HISTORY STUDIES

Especially in regions with severe climate, the mode of overwintering of parasitic fungi is of very great importance. Dudley (8), as early as 1889, in what was then the most complete work on currant anthracnose, stated that . . . "the biological investigation of this (the fungus) presents peculiar difficulties for we have little doubt that it hibernates elsewhere than on the fallen leaves of the previous season." Stewart and Eustace (29) twelve years later first reported the occurrence of the fungus on young canes. Not until 1906, however, when Klebahn, by satisfactory inoculation and pure culture tests, proved the relation of *Gloeosporium ribis* to a Discomycete, was the most important method of overwintering actually demonstrated. That this stage is extremely rare or is very difficult to find is evident from the few records in literature, and so far as the writer is aware his specimens of the perfect stage are the only ones that have been collected in this country.

Several careful examinations were made of leaf remnants in the Ribes plantation at Madison in the spring of 1931. No ascocarps could be found. No better success was had in observations at Sturgeon Bay the same spring. That fall, in Madison, large quantities of heavily infected currant leaves were picked and placed in cloth netting bags on sod for overwintering. By February only a few pieces of petioles remained. Throughout the winter, leaves had been gathered in the currant planting and a series of fixations made. By the middle of April, 1932, the leaves had so disintegrated and disappeared that no more material was available.

The first *Pseudopeziza ribis* fruiting bodies seen by the writer were found June 12, 1932, on overwintered leaves of wild black currant (*Ribes americanum*) at Sturgeon Bay, Wisconsin. Only a few ascocarps were found and no studies other than fixations were possible. On cultivated red-currant leaves mature ascospores were found on material collected May 12. Fruiting bodies were very rare in this material, which had been overwintered in cloth-netting bags on sod.

Again in October, 1932, infected leaves were placed in cloth-netting bags and in open wire pens for overwintering studies at Madison. On February 9, 1933, some leaves were collected and placed in moist dishes and incubated at 8° and 12°. They were not observed until March 16, when abundant ma-

ture ascocarps were found. Spores were discharged on potato-dextrose agar for isolation and pieces of leaf tissue were fixed. Numerous other collections were made at various intervals until May 1, forcing out the ascocarps for use in this study. On April 10, 1933, some gooseberry leaves that had been collected in the garden produced mature ascocarps after having been held for 2 weeks at 12°.

After returning to Sturgeon Bay in May, 1933, the writer resumed observations on the same Ribes plants as before. No perfect stage was found on the wild black currant in 1933. Although most of the cultivated currant leaves were decayed, there were abundant ascocarps on the leaf fragments. It was surprising, indeed, to find the gooseberry leaves well preserved and with abundant apothecia (Plate II, O). Large collections were made, isolations secured and material fixed. The ascospore-discharge studies, shown in figures 5 and 6, were made with leaves of this material. A small package of leaves was gathered May 15, stored out of doors, and, on May 27, mailed to Madison to be placed at 4°. On February 7, 1934, some of these leaves were moistened and placed over currant and gooseberry bushes in a moist chamber, and abundant infection resulted from the ascospores discharged. On April 17, these ascospores were still highly viable. At the last test, December 2, 1934, the spores discharged readily but very few germinated.

In the fall of 1933 diseased currant leaves of the Franco-German variety, considered highly resistant to this fungus, were collected and laid down as usual. Throughout this investigation leaves of this variety have proved to be the most favorable for overwintering studies. Collections of this material were made at intervals beginning in December and placed in moist dishes at 8° and 12°. The leaves collected January 13, showed ascocarps that contained mature spores after 30 days at 8°. The best temperature for maturing the perfect stage depends on the time of collection or stage of fungus development. For early material in January and February, 8° is best and later 12–16–20° hasten ascocarp maturation. This organism appears to have a graduated thermal requirement for ascocarp development somewhat similar to that described by Wilson (33) for *Venturia inaequalis*. If the leaves are kept at 22° or above, few ascospores are formed but, rather, conidia or sterile ascocarps. That these semi-natural conditions are favorable for ascocarp development is shown in plate II, P.

During the winter of 1933–34 fixations were made of heavily infected Franco-German leaves wintering under natural conditions. Collections were made about the middle of each month beginning in December. All material was fixed in F. A. A., inbedded in paraffin, sectioned 8–10μ thick and stained with safranin and fast green. For comparison, the series prepared in 1931–32 was reexamined and found to agree with the one of 1933–34. To show certain stages in the development of the ascocarps on Franco-German leaves photomicrographs are presented in plate III, A–H.

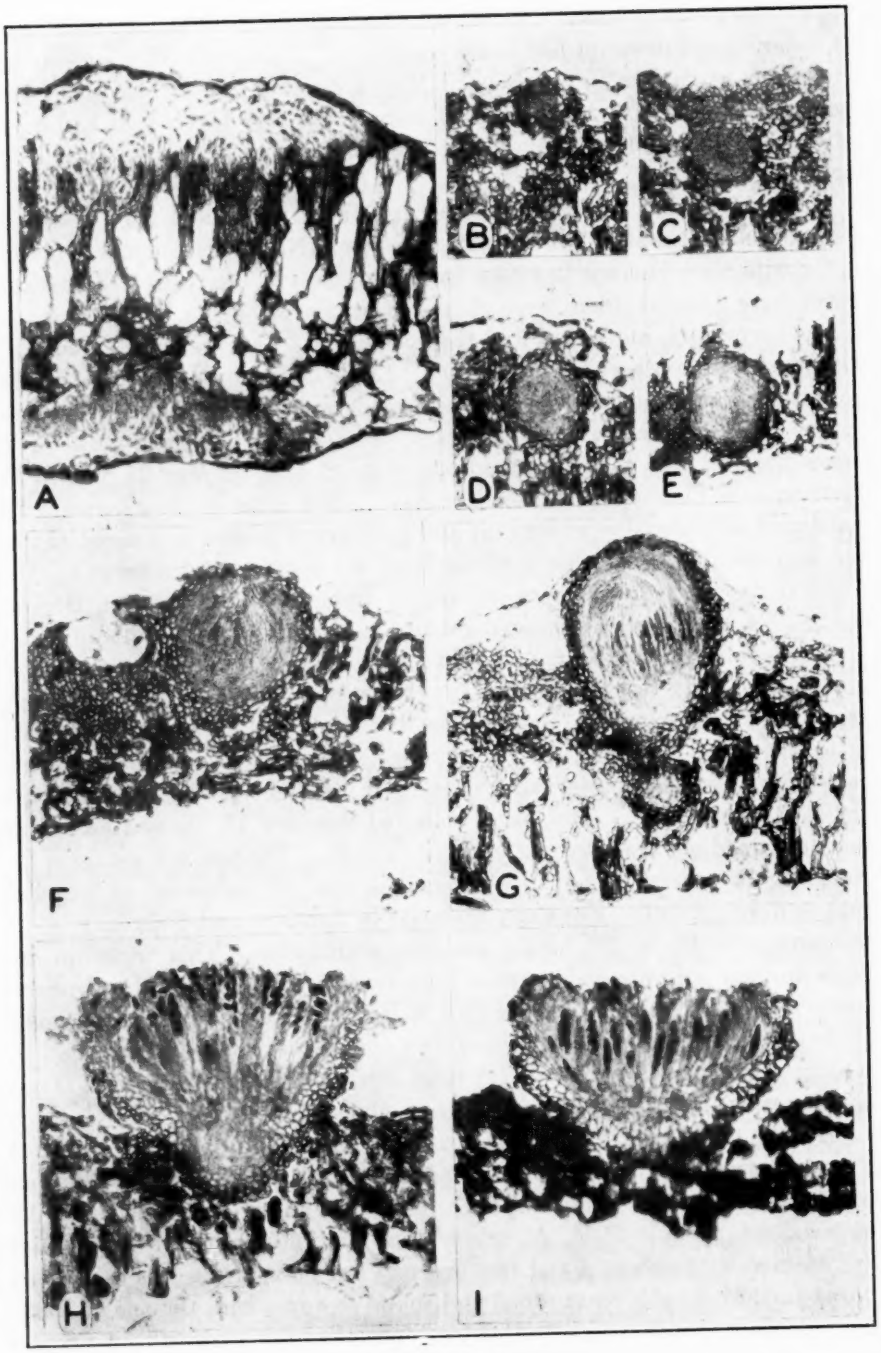

PLATE III

At the close of the vegetative period of Ribes plants the fungus in diseased leaves produces large numbers of conidia and microconidia. These may be borne together or in separate acervuli (Janczewski and Namyslowski (14)). The mycelium is mainly hyaline and fine and is largely restricted to the lesion. Later in the season, especially after the leaves have fallen, the fungus apparently enters a saprophytic stage and the mycelium extends in the tissues and enlarges, its walls become thick, and heavy mats of pseudoparenchymatous tissue are formed beneath the acervuli (Plate III, B). The initials of the fruiting body may arise in this mass (Plate III, C), or elsewhere along the extended mycelium as small groups of twisted hyphae. Unfortunately, the morphological and cytological details of ascocarp development are not known; therefore, it remains to be determined whether or not there are sexual processes involved. The ascocarp initials are formed at varying depths in the tissue of the leaf, and as they enlarge and mature they approach the surface, finally emerging in a typical, flat-top, disc-shape apothecium, sessile or slightly stalked. Normally the fruiting bodies will mature only on the lower leaf surface, although occasionally they occur on the upper.

Small portions of blades and of petioles or entire leaves protected in the crown of the bushes may provide ascosporic inoculum for primary infection. In nature, ascospores were mature on Franco-German leaves at Madison about April 27, 1933, and at Sturgeon Bay on cultivated gooseberry leaves about May 12. The records thus show that mature spores are present about the time the bushes foliate. That these very young leaves are susceptible was shown by an experiment described earlier in this paper.

Primary infection in the spring may be initiated by ascospores, by conidia formed in the spring, and probably by conidia formed in the fall. The production of ascospores has been described. These spores are discharged only when the leaves are moistened with rain or dew and may be carried by air movements to susceptible parts to which they adhere very tenaciously. At temperatures of 16–22° in the greenhouse infection appears in 8–9 days after inoculation with ascospores.

PLATE III. Stained sections of Franco-German leaves, showing certain stages in the development of the ascocarps of *Pseudopeziza ribis*. ×165. A. Collected just previous to leaf fall, October 11, 1932, showing acervuli with spores on both leaf surfaces. Microconidia are present but do not show here. B. Collected December 13, 1933, showing extensive development of the heavy-walled mycelium and an apothecial initial. C. Collected January 13, 1934. D. Collected February 12, 1934. E. Collected March 12, 1934, showing the beginning of differentiation of the contents of the fruiting body. F. Collected April 12, 1933, showing a later stage. G. Collected April 12, 1933, showing the young asci. H. Mature ascocarp with spores. There is some indication of impoverishment of the mycelium in the host. I. Mature ascocarp on gooseberry leaf.

Conidia may be formed in abundance on the overwintered leaves. Klebahn (20) assumed that the fungus on gooseberry overwintered in this manner instead of by ascospores. The writer has found conidia being produced on overwintered gooseberry and currant leaves either in stromatic acervuli or more or less definite pycnidium-like bodies (Plate II, M). Microconidia have also been observed in abundance on overwintered leaves, but their exact origin whether in acervuli, ascocarps, or pycnidia, was not determined. That conidia produced on overwintered leaves are able to infect green leaves, was shown in a test on April 12, 1933, whereby excised Downing and Red Jacket leaves were inoculated in a Petri dish at 22° with spores from overwintered gooseberry leaves that had been kept at 12° for several days. This experiment gave abundant infection within 7 to 8 days. It is highly probable that these conidia may prove a source of primary inoculum.

That conidia of *Pseudopeziza ribis* would remain viable over winter, was first reported by Ewert (12). He did not secure infection with them, however. The writer has on several occasions secured germination of conidia from overwintered leaves taken directly from the field, but has no proof that they will infect leaves. Abundant spores on leaves in midwinter after warm periods indicate that spores may be formed at any time the temperature is above freezing. Thus spores found on overwintered leaves may have been formed only a short time previously.

During the summers of 1931 and 1932 at Sturgeon Bay, the writer made frequent examination of a currant planting and kept general records of disease and host development. These records agree in general with the data presented in detail for 1933 and 1934. The particular planting observed was composed of 39, 20-year-old gooseberry, 35 Cherry currants and 2 white currant bushes, all poorly cared for by the owner. It was approximately 3 miles from the station where weather data were recorded. Notes on host development and progress of the disease, including defoliation and leaf spotting, were followed on 10–20 shoots of 2 nonsprayed bushes of each variety. The ascospore discharge record was taken on 5 gooseberry leaves by the method used by Keitt and Jones (16). It consisted essentially of placing a small glass slide smeared with glycerine jelly about $\frac{1}{8}$ inch above each leaf, which rested flat on the ground. Each day at 8 A. M. the slides were changed and the spores counted. The temperature, rainfall and humidity records were taken from continuously recording instruments. Special attention is invited to the data relating to epidemiology presented in figures 5 and 6. The comparatively early and severe outbreak of the disease in 1933 may be correlated with the abundance of early primary inoculum and the occurrence of favorable, moist weather conditions. In 1934, after a slow start, the disease became extremely severe and spread rapidly late in the season.

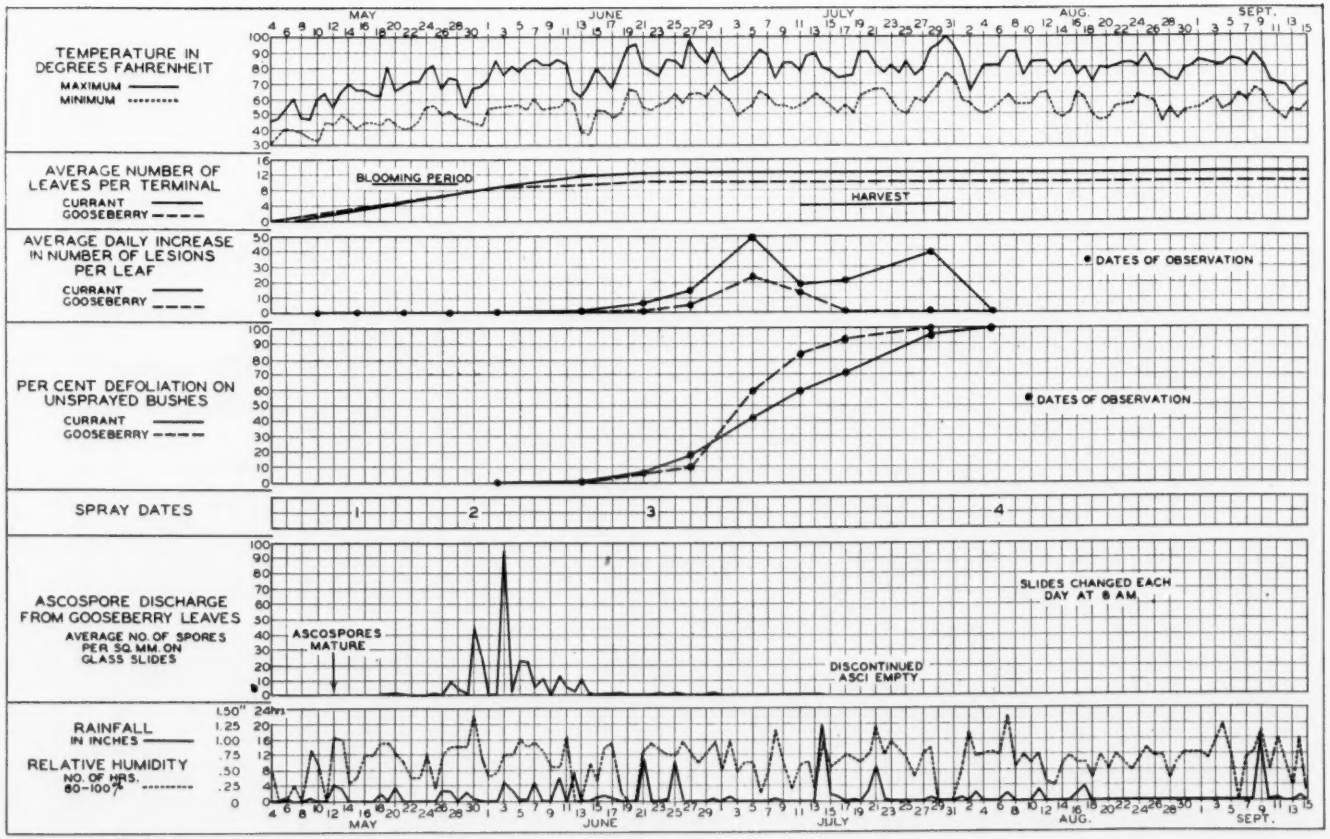

FIG. 5. Graphic representation of several factors important in the epidemiology and control of currant and gooseberry anthracnose, Sturgeon Bay, Wisc., 1933. (See table 8 for results of spray treatments and text for further explanation.)

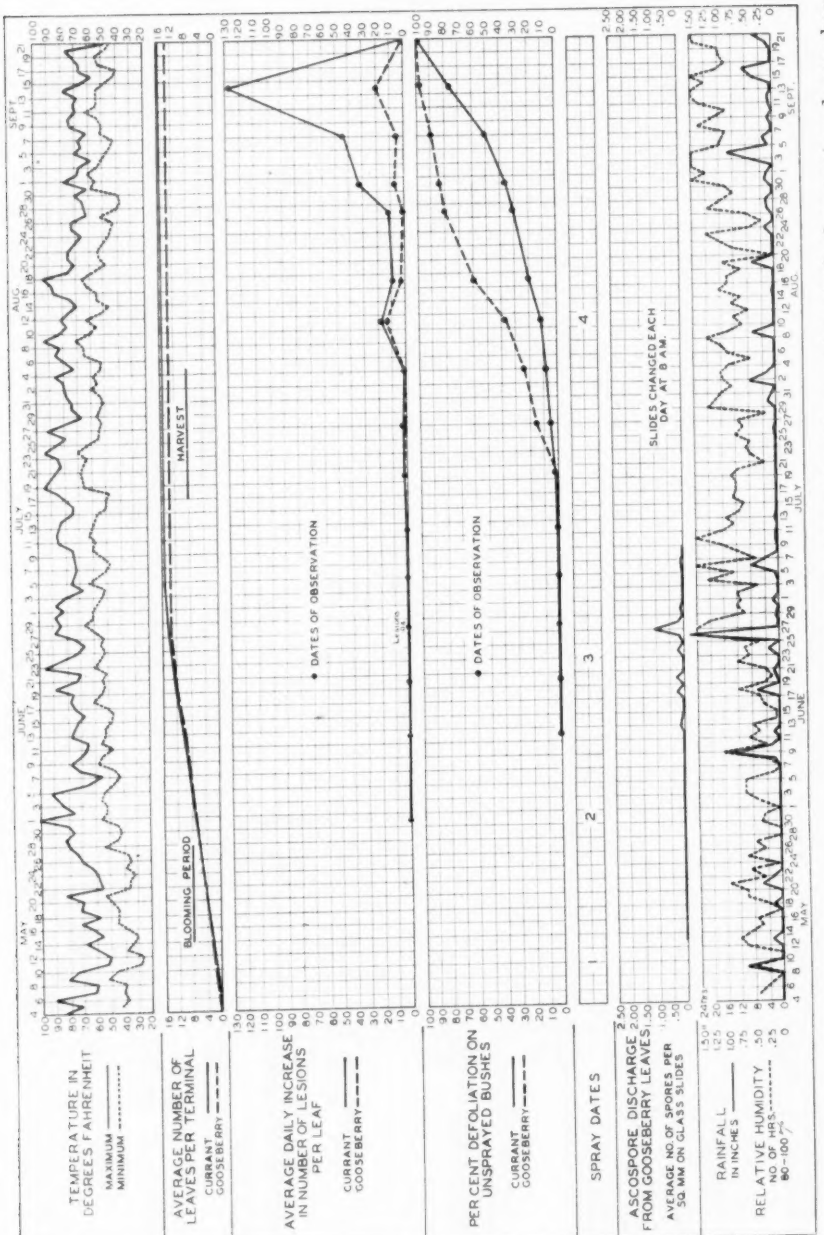

FIG. 6. Graphic representation of several factors important in the epidemiology and control of currant and gooseberry anthracnose, Sturgeon Bay, Wisc., 1934.

CONTROL

Contributions to the development of satisfactory control measures for anthracnose have been made by many workers over a wide range of conditions. Dudley (8), in 1889, suggested the use of "one of the copper solutions" if a severe outbreak seemed evident. The first experiments of consequence apparently were those by Ewert (10), in 1905. He reported the results of an extensive experiment using various copper sprays all of which gave good control. He also found that heavily pruned bushes were much less severely affected than his checks.

Ewert confirmed his conclusions regarding the effectiveness of copper sprays in very extensive experiments reported in 1907 (11) and further trials in 1913 (13). Noffray (28) in France recommended cutting out the disease as it appeared. The experiments in England by Marsh and Maynard (24, 25, 26) on black currants mainly of the Baldwin variety show conclusively that Bordeaux mixture is very effective in controlling anthracnose. Further striking proof is given of the value of maintaining vigorously growing plants through adequate nutrition and by pruning methods.

In the United States, Stewart (30, 31), working with nursery stock, found lime-sulphur and Bordeaux mixture to be effective if applied at the proper time. Dutton (9), after extensive trials, concluded that Bordeaux mixture, lime sulphur, and dusting sulphur were satisfactory, but the former was more efficient. Caesar (3), in Canada, reported good control with Bordeaux mixture, less satisfactory control with lime-sulphur and poor results with soluble sulphur and copper-lime dust.

Since the discovery by Klebahn (18) of the fruiting bodies on overwintered leaves, destruction of the old foliage is generally recommended.

Spraying experiments for the control of anthracnose were conducted by the writer in 1932, 1933, and 1934 on the planting at Sturgeon Bay in conjunction with life-history studies reported earlier in the paper. In 1932 the experiment was on a small scale, involving the use of a knapsack sprayer. Very good results were secured in a program similar to that of 1933 and 1934 with the Bordeaux mixture and lime sulphur.

The treatments in 1933 and 1934 were applied with a barrel pump to the whole planting, except 2 currant and 2 gooseberry bushes for checks. Generally 5 or more bushes of each variety were sprayed when the full spray schedule was followed, while only 1 or 2 bushes were treated with each of the reduced programs. The materials used, dates of spray application and comparative results of the treatments in 1933 are shown in table 8. In this table also are given the data on control of the gooseberry powdery mildew (*Sphaerotheca mors-uvae* (Schw.) B. and C.). In these tests and in 1934, lime sulphur provided complete protection from mildew, while Bordeaux mixture gave quite poor control of this disease. Where the two diseases are

TABLE 8.—*Relation of spray treatments to control of currant anthracnose and gooseberry powdery mildew, Sturgeon Bay, Wisconsin, 1933*

Spray treatment[a]	Fruits mildewed on June 27[b]	Approximate defoliation on Aug. 5	Defoliation on Sept. 14
	%	%	%
Currant			
B. M., 1, 2, 3, 4	.....	0	23.1
do. 2, 3, 4	.....	5	32.1
do. 1, 2, 3	.....	0	38.9
do. 2, 4	.....	55	97.1
Nonsprayed	.....	100	100.0
L–S., 1, 2, 3, 4	.....	5	31.4
do. 2, 3, 4	.....	10	50.8
do. 1, 2, 3	.....	5	85.9
do. 1, 2, 4	.....	10	58.5
do. 2, 4	.....	90	100.0
Gooseberry			
B. M., 1, 2, 3, 4	61	0	28.7
do. 2, 3, 4	67	20	39.8
do. 1, 2, 3	61	0	52.2
do. 1, 2, 4	68	20	87.5
do. 2, 4	63	85	96.8
Nonsprayed	90	100	100.0
L–S., 1, 2, 3, 4	0	5	81.8
do. 1, 2, 3	0	5	98.0
do. 2, 3, 4	28	93	100.0
do. 2, 4	34	98	100.0

[a] B. M. = Bordeaux mixture, 3–4–50, using high-calcium hydrated lime.
L–S = Liquid lime sulphur, 1–40.
Spray dates: 1, May 15; 2, May 30; 3, June 22; 4, August 6.
Arsenate of lead, 1–50, was used in sprays 1, 2 and 4.

[b] Only sprays 1, 2 and 3 had been applied at this time, and probably spray 3 had not yet influenced the counts at all.
Data were taken on 100–600 fruits per treatment.

present in the same planting, and because of danger of lime-sulphur injury, a mixed spray program is recommended by Lovett and Barss (22). In 1934, at Sturgeon Bay, a similar schedule proved very satisfactory. The first two sprays of lime sulphur for gooseberry mildew were followed the rest of the season with Bordeaux for anthracnose. Convincing evidence was found that foliage of weak gooseberry bushes was severely burned by lime-sulphur sprays during hot weather, while healthy bushes close by showed no injury.

Results from the spray programs tested during the 3 years indicate that Bordeaux is more efficient than lime sulphur in controlling anthracnose. The Bordeaux treatment before bloom is the least important.

The Bordeaux program for currants and the lime-sulphur-Bordeaux schedule for gooseberries, with thorough application of spray, combined with proper cultural methods, may be expected to give satisfactory control of anthracnose and mildew under similar conditions.

SUMMARY

A study has been made of currant and gooseberry anthracnose, caused by *Pseudopeziza ribis* Kleb., considered the most wide-spread and destructive disease of cultivated Ribes.

Isolates of this fungus from Wisconsin, Oregon, Canada, and a culture from Europe have been studied. These showed rather wide, consistent differences in several morphological and physiological characters. All isolates studied proved to be pathogenic, but in different degrees. A method was used for measuring curved spores, and spore measurements for the several isolates are presented.

The optimal temperature for germination of conidia was about 20°, for ascospores about 12°, and for growth of the fungus on solid media about 20°. The isolates varied slightly in optimum temperatures and greatly in rate of growth. A temperature of 32° killed spores and mycelium in culture. Conidia were produced in greatest numbers at about 20–24° and microconidia most abundantly at about 8–16°. Ascospores were discharged from leaves at 1–32°.

The optimal pH for growth under the cultural conditions studied was between 5.4 and 7.0, and approximately the same for germination of conidia. Conidia were produced in greatest numbers at pH 4.0 and microconidia were produced abundantly throughout the range supporting growth.

The pH of freshly macerated currant and gooseberry leaves tested on three dates varied approximately from 5.0 to 7.0.

Infection studies were conducted on several varieties of currants and gooseberries, using conidia of all 11 isolates and ascospores from currants and gooseberries. The spores from currants were more pathogenic on currants than on gooseberries and *vice versa*. Plants inoculated on the lower leaf surface were more heavily infected than those inoculated on the upper surface. Plants held at higher temperatures prior to inoculation were more susceptible to the disease than those held at lower temperatures. Infection occurred at favorable temperatures with a moist period of 12 or more hours after inoculation, and approximately 20° was the optimal temperature for infection.

The mode of penetration, whether from conidial or ascosporic inoculation, was directly through the cuticle and the epidermal cell wall of either epidermis. Appressoria often functioned in this process. Both inter- and

intracellular development involved all leaf tissues and disorganized and killed the cells.

Development of the fungus during the fall, winter and early spring has been followed in sectioned and stained preparations and by field observations. Primary infection in the spring is initiated by ascospores, by conidia formed in the spring, and probably by conidia formed in the fall. Ascospores may infect the host as soon as the leaves unfold in the spring.

A method was developed for handling overwintering Franco-German leaves, whereby mature ascospores were available by March 1. Experimental work was greatly facilitated by the use of viable ascospores from gooseberry leaves stored at 4° from May, 1933, to April, 1934. The perfect stage on *Ribes grossularia* is reported for the first time.

A graphic summary of data on host, fungus and disease development in relation to meteorological records at Sturgeon Bay is given for 1933 and 1934.

The writer's studies and evidence recorded in the literature show that no definite statement on varietal resistance can safely be made at present. Their comparative susceptibility depends largely on environmental factors.

Experiments at Sturgeon Bay during the seasons of 1932, 1933, and 1934 indicate that Bordeaux mixture, 3–4–50, will give satisfactory control of anthracnose under the conditions encountered if thoroughly applied: (1) just before the plants bloom, (2) just after fruit set, (3) three weeks later and (4) just after harvest. Lime sulphur, 1–40, in the same program was less effective for anthracnose but highly efficacious in control of powdery mildew of gooseberry. A mixed-spray program, in which lime sulphur was applied for the first 2 sprays and Bordeaux mixture for the later ones, gave good control of both anthracnose and mildew.

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THE BIOLOGY OF GANODERMA LUCIDUM ON ARECA AND COCOANUT PALMS

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INTRODUCTION

The paucity of literature on tree and wood decay in India is probably due to the absence here of a serious factor like the *Hausschwamm*, or dry rot of Europe and America. Of late, however, the question of the rot of railway sleepers and wooden posts for electric transmission lines is attracting some attention.

HISTORICAL

Bose (6) has studied some of the wood rots in forest areas in Bengal. Butler (10) refers to a disease, "Betelnut Plague," in Sylhet, apparently caused by *Fomes lucidus*. The earliest reference to the betelnut plague is by Watt (46), who says that it was reported to him in March, 1896; he records evidence to show that it appeared in November, and December, 1892. Petch (31) states that Berkeley's record of *Polyporus lucidus* (Leys.) Fr. is from Gardner's collection of 1846 from Ceylon. Since the conditions in India are not far different from those in Ceylon, it may be presumed that this fungus was present in India at about the same time. Petch (31) collected *Fomes lucidus* (Leys.) Fr. from 1909–1915 from various parts of Ceylon. In 1913 Butler (13) refers to the finding of *Polyporus* (*Fomes*) *lucidus* on diseased areca and cocoanut palms in Mysore and Ceylon.

The first reference to the disease on areca palms, *Areca catechu* L., in Mysore, "Anabe roga of supari" (the arecanut is also known as betelnut or supari), is by Coleman (17). He obtained cultures of the fungus by placing sterile bits of diseased stem in Roux tubes with distilled water, and after the mycelium had grown out, transferring the pieces to Erlenmeyer flasks containing ground areca root dust moistened with plum decoction. He got fructifications in some of the flasks that had typical basidia and basidiospores. He did not culture the fungus on agar. A few experiments on the control of the disease in the field by trenching and liming were tried, but with no success. Work on this disease was started by the writer in the year 1928, with a view to obtaining some insight into the life history of the fungus and to utilize it, if possible, in the control of the disease.

"Anabe-roga" literally means a disease caused by "Anabe" or a mushroom-like structure, which term is applied indefinitely to any fair-sized fungus. In this particular instance, the fungus is *Ganoderma lucidum* (Leys.) Karst.

HOSTS AND SYNONYMY

The fungus *Ganoderma lucidum* was first described under the name *Fomes lucidus* (Leys.) Fr. It is largely a tropical fungus, but it occurs also in the temperate regions. This fungus or one or other of its synonyms occurs in India, Burma, Ceylon, the Federated Malay States, the Dutch East Indies, New Guinea, Africa, and the West Indies in the tropics, and in the temperate regions of Europe and the United States of America. Butler (12) has described the fungus in detail. Butler and Bisby (15) cite as synonyms:

Fomes lucidus (Leys.) Fr., *Polyporus lucidus* Fr., *Ganoderma resinaceum* (Boud.), *Polystictus egregius* Massee, n. sp. (?), *Ganoderma amboinensis* (Lam.) Pat., *Fomes amboinensis* (Lam.), *Polyporus amboinensis* Fr.

According to Van Overeem (42), the following are synonymous with this fungus:

Ganoderma sessile Murrill, *Polyporus fulvellus* Bres., *P. resinosus* Schraeder, *P. curtisii* (Berk.) Murrill (after van der Bijl), *Ganoderma mangiferae* (Lév.).

The list of hosts recorded for the fungus from various parts of the world comprises about 44 species belonging to 34 genera from 18 families. Out of these the family Leguminosae alone is represented by about 11 genera and 17 species; so that Novell (29) is partly justified in saying that "there is some cumulative evidence of its parasitism on leguminous trees." Sharples (34) has recorded *Polyporus ostreiformis* on arecanut and cocoanut palms in Malaya, and McRae (27) has found the same fungus on arecanuts in Calcutta. In Mysore the writer has noticed *Ganoderma lucidum* on *Areca catechu, Cocos nucifera, Cassia siamea,* and *Pongamia glabra.* An exudation, similar to that that takes place in these hosts affected by the fungus also has been noticed in *Artocarpus integrifolia.* No sporophores were obtained, and no determinations could be made. This study is chiefly on *Ganoderma lucidum* occurring on *Areca catechu* Linn. in Mysore.

ECONOMIC IMPORTANCE

The disease is found to be rather severe in gardens where the cultivation has been neglected, or where there is a tendency for water to stagnate due to deficient drainage. In one case it was very severe in a garden where *Saccharum spontaneum* was allowed to grow rather wildly. Watt (46) also has noticed the betelnut plague to be severe in neglected gardens. The disease usually attacks trees from about 10 years of age and upwards.

Younger trees have not been noticed to suffer from it. The losses from this disease have not been correctly estimated. From reports given by the garden owners, it is estimated that about 15 to 20 trees die annually in an acre of 400 trees. If not attended to properly and if regular replantings

are not made, the annual losses may mount up to 50 to 60 trees per acre, until whole gardens are wiped out. Butler (10) says that "the actual loss suffered amounts in many cases to more than Rs. 75/– an acre annually, while some gardens were seen in which fifteen-sixteenths of the trees had been killed."

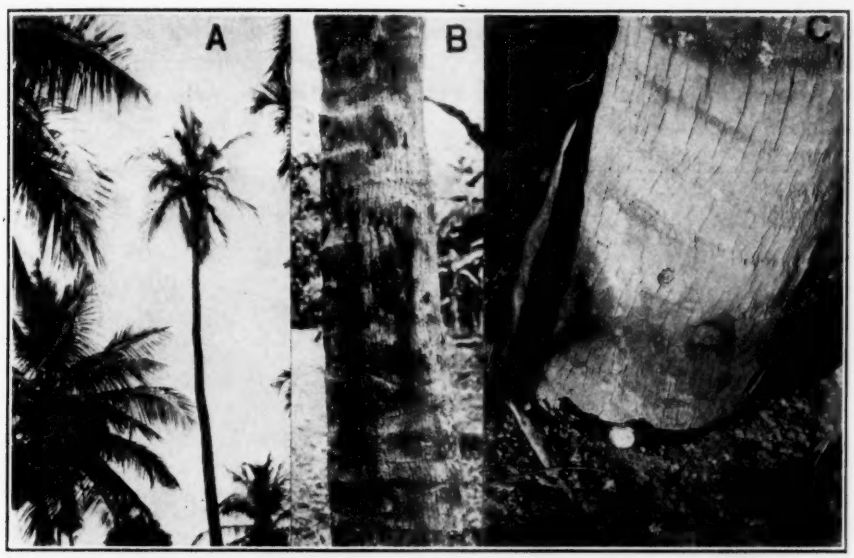

FIG. 1. A. Cocoanut palm showing narrowing of the crown and drooping of the basal leaves, the effect of ganoderma infection; healthy palms on either side. B. Another cocoanut palm trunk showing the profuse bleeding from numerous spots due to the same infection. C. Cocoanut palm inoculated with pure culture of *Ganoderma lucidum* on cocoanut showing bleeding 6 months after inoculation. The hole was plugged with cork after inoculation. The watch lying on the ground indicates the size of the inoculation hole.

SYMPTOMS

The symptoms of the disease on areca palms have been defined by Watt (46), Butler (10), and Venkata Rau (43), and for the disease on cocoanuts by Petch (32) and the writer (44). They are akin to those of drought. There is first a drooping or yellowing of the lower leaves, followed sometimes by a reduction in the size of the crown (Fig. 1, A). In later stages the crown dries up. In contrast to this, Watt (46) found the innermost leaves to be affected first, the crown of leaves separating from the trunk, and a foul smell in the bud. The ultimate effect, according to him, was to leave dead stumps behind until "what was once a plantation looked like a harbour with thousands of masts." Again, in contrast to the writer's experience, Butler (10) states that one of the earliest signs of this disease is a dropping of the

nuts, as in the Mysore disease, referring to "Koleroga" due to Phytophthora described earlier in his paper. The experience in Mysore is that affected trees continue to bear a crop for some years, even though it may be slightly less than normal. The observations of Watt and Butler lead one to suspect that they perhaps had also under notice some cases of top rot due to *Phytophthora arecae*. Indeed, Butler (10) says "the final appearance is just the same as in Koleroga." Additional support for this view is lent by the fact that Watt (46), himself, cites N. G. Mukharji's report, which records the latter's finding of a species of Pythium in diseased palms, though they could not get it a second time.

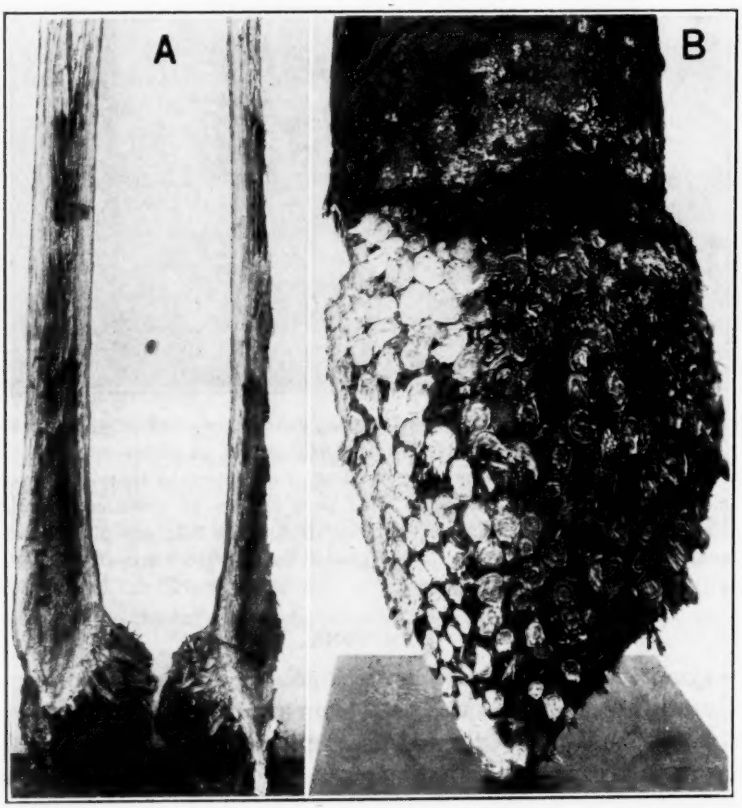

FIG. 2. A. Stump of areca palm cut open, showing late stage of ganoderma infection. The roots are all affected. B. Stump of areca palm showing the browning of the roots (right half) at an early stage of infection. Those on the left are yet unaffected.

The seat of the trouble, however, is at the base of the trunk and the roots. Affected roots are discolored, dry, and brittle (Figs. 2 and 3). From the lower portions of the trunk there is an exudation of a brownish gummy juice

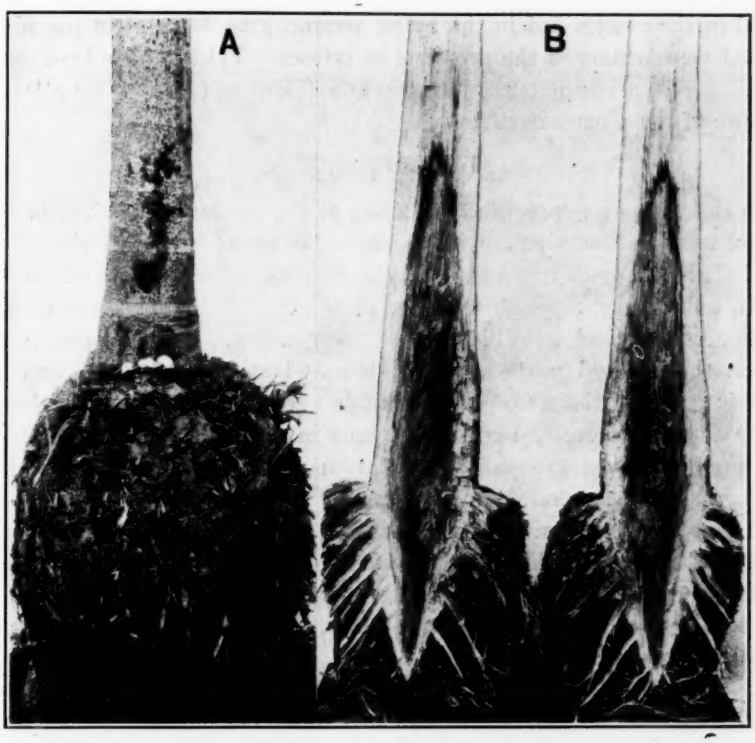

FIG. 3. A. Stump of areca palm showing the characteristic bleeding, and three sporophores at ground level. B. Stump of areca palm cut open to show the rotting inside. (Earlier stage than in figure 2, A.)

(Fig. 3, A), which is more copious in the cocoanut (Fig. 1, B) than in the arecanut. Some time after the exudation starts, fruit bodies of the fungus begin to develop (Fig. 3, A). Sometimes the fructifications appear on the stumps only after the cutting down of the palms. No stalk has been noticed in the fructifications on either the arecanut or cocoanut palms.

On cutting open an affected areca palm, the interior of the stem is found to be dark brown to about 2 to 3 feet from the ground (Fig. 3, B). The affected tissues emit a musty smell, very commonly associated with freshly growing sporophores of the Polyporaceae. On the outer border of the brownish zone is a yellowish zone, beyond which is the apparently healthy white portion. The brownish discoloration also extends down into the central thickened portion of the root system, and into the affected roots (Figs. 2, A, and 3, B). They are sometimes mottled with white on a brownish background.

On microscopic examination of the stem, the fungus was found both in the brownish and the yellowish regions, but not in the white region. It was

noticed in the vessels and in the xylem parenchyma, but not in the phloem. Affected vessels showed the presence of tyloses. Tyloses have been seen in plague-affected areca palms by Watt (46) and Butler (14). Watt attributed the cause of the disease itself to tyloses.

SECONDARY ORGANISMS

Besides *Ganoderma lucidum*, in many of the gardens visited, some of the affected trees showed signs of infection of the stems by *Thielaviopsis paradoxa*. This was evidently a saprophyte, as in the cases noticed it was found to gain entry only through wounds. Instances of trees with Ganoderma infection, alone, and no Thielaviopsis were many. Thielaviopsis appears soon on the exposed parts of palm stems. Butler (11) has found Thielaviopsis on palm tissues of several kinds very frequently, and is inclined to view it as a perfectly harmless fungus in India. Sundararaman *et al.* (40) have described successful inoculation experiments with *Thielaviopsis paradoxa* on areca palms, but only through wounds, and are themselves inclined to believe it is only a facultative parasite. Thompson (41) says in regard to the oil palm, *Elaeis guineensis*, that Thielaviopsis is the commonest mould, appearing in 36 hours on freshly pruned leaf bases, and that it is always present on diseased palm tissue, and, under certain circumstances, is definitely parasitic.

MATERIALS AND METHODS

The materials for this investigation were obtained from Amrutur, a village about 56 miles from Bangalore, where the arecanut is grown as a crop in gardens irrigated in summer from a tank.

Cultures of the fungus were obtained by transferring aseptic pieces of the tissue from near the margin of the apparently healthy and infected tis-

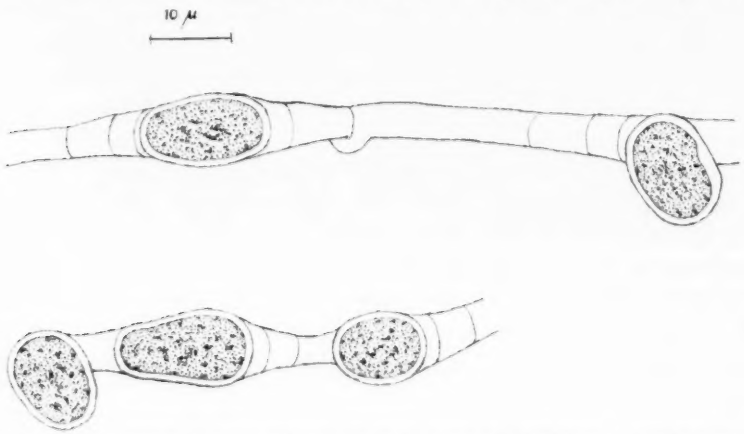

FIG. 4. Chlaymdospores in pure culture on malt agar of *Ganoderma lucidum* on areca.

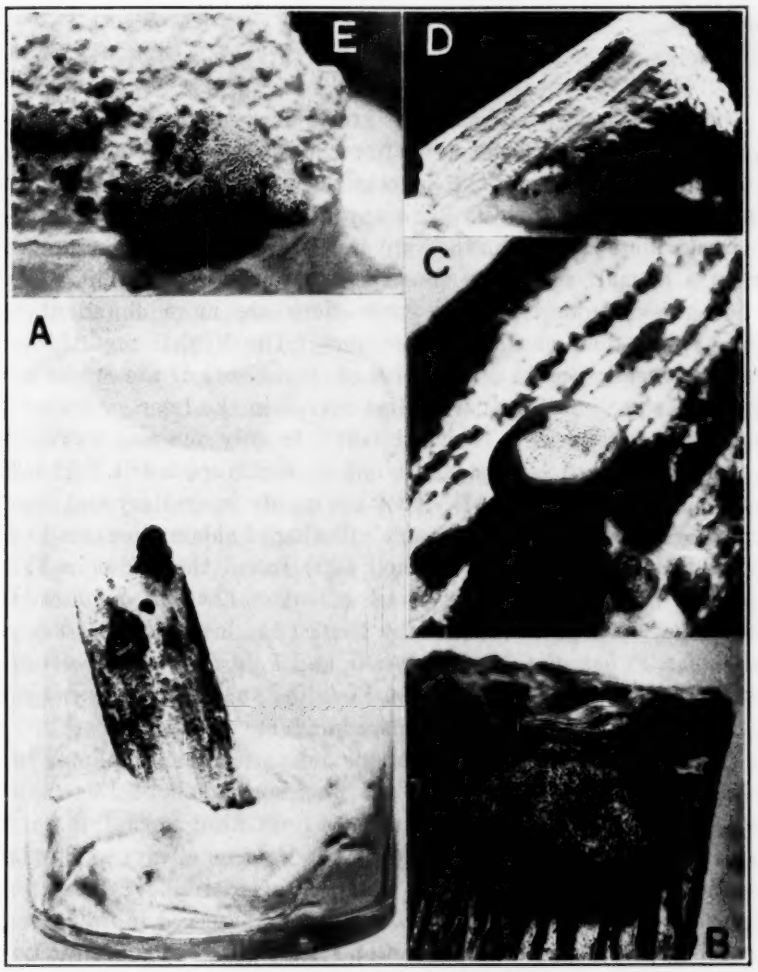

FIG. 5. A. Culture on areca wood of *Ganoderma lucidum* on areca, in bottle. Note thick mycelial mat on the wood, and spores on the mat dropped from the sporophore just under the pores. B. Another sporophore from culture on wood of the areca strain in bottle, showing the pores. C. Resupinate sporophores in culture on areca wood of the areca strain in bottle. D. Culture on cocoanut wood of *Ganoderma lucidum* on cocoanut, showing numerous origins of sporophore in the form of poriferous layers. E. Some poriferous layers from D enlarged.

sues into prune-juice agar and malt-agar slants. On the third day a hyaline mycelium grew out of the pieces, and subsequent transfers gave pure cultures.

THE FUNGUS

Cultural Characters.—The fungus grows slowly on malt and prune-juice agar. The subcultures show any appreciable growth from the inoculum only on the 5th day. In about 2 weeks the surface of the slant is covered with a thick flocculent growth. This appearance is partly due to the deposit of calcium oxalate crystals on the hyphae. The crystals are more prominent on growths in malt agar than those in prune-juice agar. There are both thin and thick hyphae. Clamp connections are more abundant on the thicker hyphae than on the thinner ones. The hyphal mat thickens on growth on agar, and more so on pieces of stem tissue of the arecanut (Fig. 5, A). The mycelium remains hyaline except in the later stages on pieces of stem, when it takes on a brownish tint. In only one case was a slightly yellowish tint noticed on a sterile hump in a culture bottle. The fungus forms chlamydospores (Fig. 4). They are mostly intercalary and sometimes terminal. Snell (38) mentions Faull's finding of chlamydospores in *Fomes officinalis*. Besides this fungus, Snell (39) found them also in *Trametes serialis, Lentinus lepideus* and to some extent in *Lenzites saepiaria* and *L. trabea*. They have been observed by Fritz (24) in *Polyporus sulphureus, P. balsameus, P. borealis, P. schweinitzii,* and *Polystictus versicolor,* and by Brefeld (7) in *Polyporus destructor, Fistulina* sp. and *Oligoporus* sp. No oidia have been observed in *Ganoderma lucidum.*

Sporophore Production.—The fungus did not form sporophores on agar, even though grown in big tubes 7″×1″, containing about 15 to 20 cc. of medium. Neither exposing these tubes to light near a window for 1 to 2 hours daily, nor keeping them always in the light or always in the dark, in the vertical or horizontal position resulted in sporophore formation. A poriferous layer containing spores was, however, observed in the strain from the cocoanut palm, both on agar and on sterile pieces of cocoanut trunk in bottles (Fig. 5, D and E). Bose (5) succeeded in obtaining loose pores without any pilear form, but containing spores of *Ganoderma lucidum* on agar. He sent a subculture of his isolation to Dr. Coleman, which on sub-culturing did form an irregular cup-shape structure bearing the hymenium and spores, somewhat similar to the poriferous layer formed by the cocoanut strain. Bose (6) has observed such structures containing only spores and numerous crystals of calcium oxalate in *Trametes gibbosa, Polyporus adustus, P. ostreiformis,* etc.

With a view to getting the fungus to produce sporophores, the method adopted by White (48) was employed. Pieces of areca stem were used from

which the air had been driven out by alternately boiling and plunging into cold water 3 times. The blocks were about $3'' \times 2'' \times 1''$, and were placed in a slanting position in 1-litre wide-mouth bottles on wads of absorbent cotton about 1″ thick, and thoroughly moistened. The bottles were autoclaved at 1 atmosphere pressure for 20 minutes, and used for inoculation after 2 weeks. Out of 8 bottles inoculated, sporophores developed in 6. Regular sporophores developed in 3 bottles, and in a fourth the sporophore remained in an incipient stage, probably owing to the drying out of the cotton. In 2 other bottles the sporophores failed to develop spores. The sporophores were of the bracket type, only smaller (Fig. 5, A and B), as was to be expected, seeing that the available nutriment was so small. Sometimes they developed resupinate fashion (Fig. 5, C). Spores resembling natural ones dropped down to the bottom of the bottles.

In a month from the time of inoculation, drops of a colorless juice were seen on the mycelial mat covering the pieces of stem. These were sometimes seen also in the culture tubes. Bose (6) has noticed a juice in *Ganoderma lucidum* and *Trametes gibbosa*. In the latter he observed the drops to contain numerous round, white and brownish conidia, both of approximately the same size, by transferring which to agar he recovered the mycelial growth of *T. gibbosa*. No conidia were seen by the writer in the drops secreted by *G. lucidum*. The earliest signs of sporophore formation occurred on the 72nd day. Three humps were noticed that gradually developed a deep lac color, as in the natural specimens (Fig. 5, A), and in seven weeks from that time, spores were seen to shed from one of the humps at the side. In a second bottle the sporophore formed 3 days later than in the first case, pores were seen to form 2 weeks later, and spores began to fall about 5 weeks from the appearance of the pores. Hence, under the conditions prevailing in the bottles, the pores developed in about 3 months from inoculation of the pieces, and spores began to fall in about a month from the appearance of the pores.

The bottles were kept in a closed cupboard and were not exposed to light, except to the diffused light of the room for purposes of routine inspection. According to Miss Ames (1), Miss Bayliss obtained only abortive fruit bodies in the absence of light, which Miss Ames attributed to the probably abnormal laboratory conditions. White (48) failed to get the typical bracket form of fructifications in his bottle cultures. Long and Harsch (26) succeeded in getting typical pilei in artificial cultures on agar when light was made to fall parallel to the tubes irrespective of whether these were placed vertical or horizontal. They found from their study of wood rotting fungi that many *Polyporaceae* produced sporophores in diffused light, others required direct sunlight, while some produced sporophores in absolute darkness like *Polyporus farlowii, P. cinnabarinus,*

and *Trametes serialis*. *P. cinnabarinus*, unlike the other 2, produced only sporophores but no spores. They found that the character of the substratum played only a minor part in sporophore formation. Bose (6) found that the direction of the rays of light had no influence in the development of typical pilei by *Trametes lactinea*.

SPORE GERMINATION

Various media were tried for germination of spores, such as malt agar, prune-juice agar, distilled water, tap water, rainwater, 1 and 2 per cent malic and citric acids, and Buller's gelatine medium (9). In no case was germination observed. Both material from the field and from artificial culture gave negative results. Coleman (18) also failed to germinate the spores, and he believed that the failure might be due to the chitinous endospore, because spores with thin undifferentiated walls like those of *Fomes fomentarius*, germinate readily. Bose (5) got germination of the spores of *Ganoderma lucidum* on 3 per cent malt-extract agar of pH 6.9.

DECAY

Since decay is essentially an enzyme action, an attempt was made to study the enzymes present in *Ganoderma lucidum*. Buller's work (8) on *Polyporus squamosus* showed that this fungus secreted laccase, tyrosinase, amylase (diastase), emulsin, protease, lipase, rennetase, and "coagulase," and possibly cytase and hadromase, all except the last two in the sporophores.

Schmitz and Zeller (33) found that the following enzymes were present in *Polyporus lucidus* viz., esterase, maltase, lactase, sucrase, raffinase, diastase, inulase, cellulase, hemicellulase, emulsion, tannase, urease, and trypsin and erepsin when fibrin is used as a substrate.

Nutman's work (30) on *Polyporus hispidus* showed that the fungus secretes emulsin, diastase, invertase, ligninase, hemicellulase, oxidase, and catalase.

In this work on enzymes Nutman's (30) method of growing the fungus on liquid extracts and testing for the enzymes in the mycelium was followed. The extracts used were of turnip, beet-root and carrot. Two hundred and fifty grams of each sliced thin were added to 500 c.c. water and boiled in the autoclave at 115° C. for 20 minutes, cooled, filtered, each put into 5 Erlenmeyer flasks and autoclaved at 115° C. for 20 minutes. The flasks were inoculated with 4 pieces each of inoculum from malt-agar culture. Only 1 of the carrot decoction flasks got contaminated and was discarded. The surface of the liquid in the flasks of turnip extract was covered by the fungus growth in from 10–15 days, whereas that of carrot was filled in 20 days, during which time the beet-root extract was only half-covered.

Thus, as Nutman found, turnip extract is the best medium for the growth of the fungus. The cultures were kept at laboratory temperature. After 8 weeks, the mycelial mats were washed and dried between blotting paper and then over calcium chloride *in vacuo* for a week. The enzyme extract was prepared by grinding the dried mycelial mat with an equal weight of silver sand in an agate mortar and steeping it in distilled water, 20 parts to 1 of mycelium for 24 hours. The extract was filtered and toluene added as an antiseptic. The methods were adapted from Buller (8) and Nutman (30).

ENZYME TESTS

Diastase

One c.c. of the enzyme extract was added to 20 c.c. of a 5 per cent solution of soluble starch in a test tube. To another tube containing the same amount of starch, 1 c.c. of the boiled extract was added and to a third tube 1 c.c. distilled water. The three tubes were tested after a day with Fehling's solution. The first tube to which the unboiled extract was added showed reduction, while the other two did not. Hence *Ganoderma lucidum* secretes diastase.

Laccase

To 10 c.c. of a 1 per cent solution of hydroquinone in a flask 1 c.c. of the enzyme extract was added and to another flask of the same solution 1 c.c. of the boiled extract. In the flask to which the enzyme extract was added a brownish red color developed after 24 hours, which deepened after 48 hours. There was a green iridescent pellicle on the surface of the liquid, as observed by Buller (8). The control gave no change, even after 8 days. This shows that the fungus secretes the oxidase, laccase.

Protease

To a solution of 7 per cent nonneutralized gelatine in 2 test tubes, 2 c.c. of extract was added to the one and 2 c.c. of the boiled extract to the other. A little toluol was added to each tube as an antiseptic. After 24 hours the gelatine was liquefied in the first tube, but not in the second. The gelatine was liquefied to a depth of 6 mm. after 48 hours and a further 3 mm. after another 30 hours. The control was unaffected. This experiment shows that the fungus secretes a protease.

Rennetase

To 10 c.c. of fresh milk in a test tube, 1 c.c. of the enzyme extract was added and to another tube of milk 1 c.c. of boiled extract. Toluol was added to both tubes as an antiseptic. The tube containing unboiled extract

showed coagulation after 5 hours, while the control showed no change, showing that the mycelium secretes rennetase.

Lipase

To 40 c.c. of a 1.84 per cent ethyl acetate in each of 2 flasks, 2 c.c. of extract was added to one and 2 c.c. of boiled extract to another. One-half c.c. toluol was added to each flask. The flasks were corked and placed on top of a paraffin imbedding bath, where the temperature ranged from 42° C.–45° C. Ten c.c. portions were taken from both flasks after 5 days and 7 days and titrated with N/20 KoH. In both cases, on both occasions, the experimental portion required less alkali for titration than the control, instead of requiring more, which should have been the case if there had been any hydrolyzation. This shows that the fungus does not secrete lipase.

Invertase

To 8 c.c. of 1 per cent cane-sugar solution in each of 3 test tubes was added 1 c.c. of enzyme extract to one tube, 1 c.c. of boiled extract to a second, and 1 c.c. of distilled water to the third. After 24 hours there was a very faint reduction in the first tube, which increased slightly after 48 hours, but there was no reduction in the other two. But that it is able to utilize cane sugar is evident from the fact that in the solutions used in the H-ion concentration studies described later, the fungus develops a thick mycelial mat. *Ganoderma lucidum* seems to secrete small quantities of invertase, as was found to be the case by Nutman (30) in *Polyporus hispidus*.

Maltase

To 100 c.c. of 2 per cent maltose in each of two 125 c.c. Erlenmeyer flasks, 1 c.c. of extract was added and the contents of one of the flasks boiled. After cooling, 1 c.c. toluol was added to each flask and the flasks were stored at room temperature (about 25.5° C.). After 3 days 25 c.c. from each flask were removed and mixed with basic lead acetate and filtered. The clear solutions were tested in the polarimeter. The readings were the same in both solutions, indicating that the fungus does not secrete maltase.

Coagulase

A 5 per cent solution of Lintner's soluble starch was made, boiled, and filtered. The filtered solution was put into wide test tubes 7″ x 1″. To one of the tubes 1 c.c. of the enzyme extract was added, and to the other 1 c.c. of the extract, boiled. To both tubes 0.5 c.c. toluol was added as an antiseptic. After 15 hours a cloudy precipitate 1″ high was noticed at the top of the first tube, but none in the second. After about 90 hours

the precipitate gradually settled down in the first tube and the second showed no change. The next day the clear solutions were tested with iodine; that from the first tube gave a violet reaction, whereas the one from the second tube gave a blue reaction. This experiment proves that the fungus secretes coagulase.

Catalase

Presence of this enzyme was tested by adding the enzyme extract, the boiled extract, and distilled water to hydrogen peroxide. There was no frothing or effervescence in any case. This shows that catalase is not secreted by the fungus.

Oxidase

For the determination of oxidase Bavendamm's method (3) of growing the fungus in agar to which tannic acid had been added was tried. The medium was potato-dextrose agar in Petri dishes, and the percentages of tannic acid tried were 0.25, 0.5, 1, 1.5, 2 and 3 per cent, respectively. The

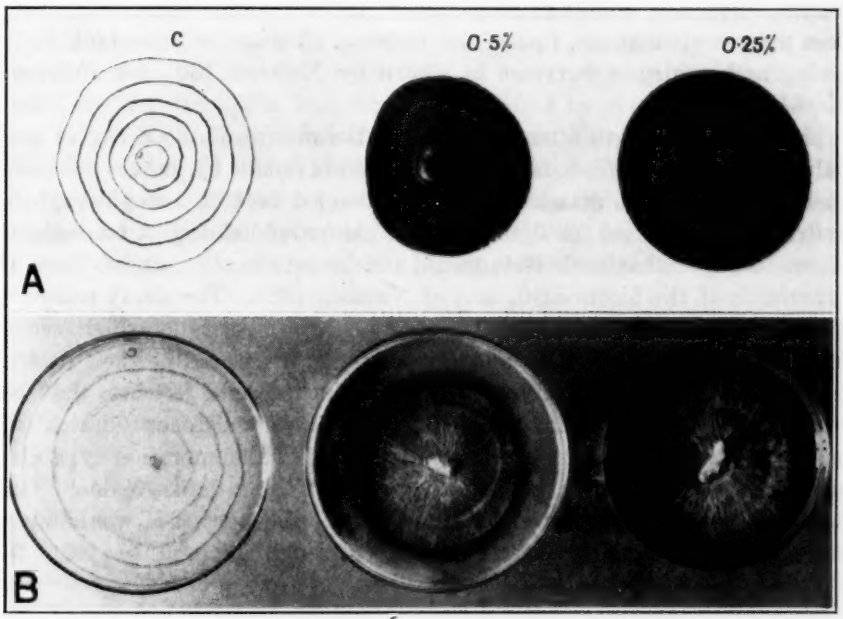

FIG. 6. Petri-dish cultures on potato-dextrose agar of *Ganoderma lucidum* on areca to demonstrate the production of oxidase. A. Bottom view of dishes showing the limit of growth from third to eighth day. B. Top view. Both series from left to right, check (c), 0.5 per cent tannic acid, 0.25 per cent tannic acid. In the latter two cultures the brown halo around the fungus is prominent and can be seen in advance of the mycelium.

agar to which 0.25 and 0.5 per cent tannic acid was added set, while the others did not. The fungus grew well in plain potato-dextrose agar, and in 0.25 and 0.5 per cent tannic acid potato-dextrose agars, but there was no growth in the others. The brown halo in the tannin cultures was quite noticeable, even on the second day and kept on increasing from day to day in advance of the mycelium. (Fig. 6). This experiment shows that the fungus secretes oxidase and is thereby able to utilize tannin. Since tannin is analogous to lignin it may be presumed that the fungus is capable of destroying lignin. Viala (45) has found that *Fomes igniarius*, causing apoplexy of the vines, destroys tannin by means of an oxidase in the dead wood, which acts on the tissues in advance of its growth. He also found that active growth occurs only in tissues rich in tannin, and the attack occurs usually on vines after they are about 15 years old, before which treatment is not required. This may possibly account for the fact that ganoderma infection has not been noticed on very young arecanut or cocoanut palms.

These enzyme studies have shown that the fungus secretes diastase, laccase, invertase, protease, coagulase, rennetase, and oxidase, and that it does not secrete maltase, lipase, and catalase. Thus it secretes both hydrolysing and oxidising enxymes as shown by Nutman (30) for *Polyporus hispidus*.

In the early stages attempts to study the nature of the decay in areca palms attacked by *Ganoderma lucidum* were made by means of microchemical tests. The attacked tissues responded both to the phloroglucin-hydrochloric acid test for lignin and the chlor-zinc-iodide test for cellulose. It was thought that such tests would not be satisfactory, as has been the experience of the biochemists, and of Nutman (30). The decay caused by *Ganoderma lucidum* on areca palms is of the brown-rot type, which, according to Falck and Haag (23) and Bavendamm (3), would be the "destruction" type. Since *G. lucidum* can use tannin, it would fall into the "corrosion" group, unless it may be one of the transitional forms. Falck (22) has found that *Fomes annosus* causes a decay of the corrosion type characterized by the simultaneous disintegration of lignin and cellulose. Only detailed chemical analyses of the sound and decayed tissues would give a correct idea of the nature of the decay.

H-ION CONCENTRATION

That soil acidity favors the growth of wood-destroying fungi has long been recognized. Wolpert (49) has summarized the literature on this subject. He found that wood-destroying fungi grew best on acid solutions and that their growth increased the initial acidity of the solutions. Anderson (2) found that soil acidity is the dominant predisposing cause in the

attack of *Fomes annosus* (*Trametes radiciperda*). This view has been supported by Weis and Nielsen (47). Curtin (19) and Curtin and Thordarson (20) found acid production in a number of fungi. Curtin (19) opines that acids considerably assist in the liberation of carbohydrates that serve for the nutrition of wood-rotting fungi. Campbell's work (16) on the chemical aspect of wood rots has led to the conclusion that acid production and enzyme action are closely related. He suggests that acids might be formed by the action of an oxidase on lignin and pentosans and then these react together with the oxidase to bring about the later stages of decay in which cellulose is decomposed.

Wolpert's method (49) was followed to study the growth of *Ganoderma lucidum* in relation to H-ion concentration. Two culture solutions were used, modified Richard's E solution, and peptone nutrient solution with sugar. The pH readings were taken with the aid of a Hellige comparator, and only double-distilled water was used in preparing the solutions. Precautions regarding glassware suggested by Wolpert (49) were taken and pipettes were dry-sterilized at 150° C. for one hour. The cultures were kept at laboratory temperature. The mean temperature during the time of the experiment varied from 22.5° C. to 30° C. Observations were made upon the final pH and the dry weight of the fungus in the manner suggested by Wolpert. The inoculum was prepared by growing the fungus on agar according to Zeller, Schmitz, and Duggar (50), as recommended by Wolpert. To 1000 c.c. of potato water (from 200 grams of peeled and sliced potato cooked for 30 minutes in the autoclave at 15 lbs. pressure) were added 20 grams of cane sugar 10 gms. KNO_3, 5 gms. of KH_2PO_4 and 20 gms. of agar. Eight m.m.-square pieces of inoculum were used, one to each flask.

Modified Richard's E Solution

This solution contained: $MgSo_4$, 0.5 gm.; KNO_3, 5.0 gm.; NH_4NO_3, 10.0 gm.; Cane sugar, 50.0 gm.; $FeSo_4$, trace; and varying amounts of H_3PO_4, KH_2PO_4, and K_2HPO_4 to give a total of 10.4 gms. of phosphate; and double-distilled water 1000 c.c. Its composition was adjusted as recommended by Wolpert (49). A total of 35 c.c. of solution was used in 100–120 c.c. Erlenmeyer flasks. Three flasks of each grade of pH were prepared, one being used to verify the pH and the other 2 for growing the fungus in duplicate cultures. In a few instances owing to the necessity of adding the phosphate solutions after sterilization, contamination resulted in one of the flasks which was rejected, and the weight of the mycelium taken from only one of the flasks. Otherwise, the weights given are the average of the two cultures. There was a slight variation in the initial pH as given by Wolpert (49) and as ascertained by the Hellige comparator,

and to show these comparatively, Wolpert's figures are given alongside in the tables. The dry weights were taken at the end of 7 weeks from the date of inoculating the cultures.

TABLE 1.—*Showing the growth of Ganoderma lucidum in modified Richard's E. Solution of different pH*

pH			Dry wt. of mat in mgs.
Wolpert	Initial	Final	
3.0	3.4	2.8	6.6[a]
3.5	3.8	2.8	12.2
3.9	4.0	2.8	12.8[a]
4.4	4.5	2.8	8.1
5.0	4.5	2.8	17.8
5.5	5.4	2.8	34.0
6.0	6.0	4.1	23.0[a]
6.5	6.6	4.3	93.5
7.0	7.0	4.5	45.1
7.6	7.4	6.8	only very faint growth
7.8–8.2	7.8–8.2	.....	no growth

[a] Only 1 culture, the others 2.

It is seen that in modified Richard's E solution the best growth of the fungus is obtained at pH 6.5 with a weight of 93.5 mgs., with a secondary maximum at pH 5.5 with 34 mgs. of mycelium. The fungus grows at pH 3.0, and even at pH 7.0 the mycelium attains a weight of 45.1 mgs. But on the alkaline side there is a sudden drop, the fungus barely showing any growth at pH 7.4 and none at all at pH 8.0. In all the solutions the fungus increased the initial acidity.

Peptone Nutrient Solution with Sugar

This solution contained Witte's peptone, 25 gms., cane sugar, 30 gms., $MgSo_4$, 0.5 gms., $FeSo_4$, trace, varying amounts of H_3PO_4, KH_2PO_4 and K_2HPO_4 to give a total of 9.65 gms. of phosphates, and double-distilled water to make 1000 cc. of solution. The dry weights of mycelium were taken 6 weeks from the date of inoculation.

The peptone solution with sugar was the better of the two solutions used, the poorest growth in this solution being better than the best growth in modified Richard's E. solution. The fungus grows best at pH 6.6, with a weight of 300.3 mgs. of mycelium. There are secondary maxima at pH 5.1 and 4.1 with weights of mat of 235.5 mgs. and 215.1 mgs., respectively. The fungus grows at pH 2.8 with mycelium weighing 95.1 mgs. It grows at pH 7.0, but here again, as in Richard's E solution, there is a sudden decline in growth

TABLE 2.—*Growth of Ganoderma lucidum in peptone nutrient solution with sugar of different pH*

pH			Dry wt. of mat in mgs.
Wolpert	Initial	Final	
2.0	2.0	2.0	no growth
2.5	2.8	2.8	95.1
3.0	3.7	2.8	157.4
3.5	4.0	2.8	202.9[a]
3.9	4.1	2.8	215.1
4.5	4.5	2.8	150.4[a]
5.0	5.1	2.8	235.5
5.6	5.7	2.8	159.0
6.0	6.2	6.0	169.0[a]
6.5	6.6	5.5	300.3[a]
7.0	7.0	6.0	130.2[a]
7.4	7.7	7.6	trace[a]
7.8–8.2	7.9	7.7	trace[a]
8.5–8.7	8.5	8.5	no growth[a]

[a] 2 cultures, the others 3.

on the alkaline side. In peptone solution also the fungus tends to increase the initial acidity. Wolpert (49) failed to notice an increase in acidity in this solution for the fungi he studied, except for *Lenzites saepiaria,* which alone increased the acidity throughout the entire pH range and at all temperatures.

The tendency noted by Wolpert (49) of a wider optimum range of pH in the more favorable solution, with a slightly fluctuating optimum zone covering several pH units is noticed also for this fungus. In the peptone solution the fungus shows good growth between pH 3.0 and 6.5, whereas in Richard's E solution the range is only between pH 5.0 and 7.0. In the case of Richard's E solution the extreme limit of the acid scale was not determined. This, in the peptone solution, is pH 2.0, which does not support the growth of the fungus. On the alkaline side pH 7.6 does not support growth in either of the two solutions.

It is clear that *Ganoderma lucidum* secretes acids during its growth.

PARASITISM

The bracket fungi have been considered to be wound parasites. The inoculation experiments with fungi of this type on living trees have not been very successful. White (48) has reviewed the literature on parasitism of the wood-destroying fungi. Since then, Baxter (4) and Nutman (30) have obtained some amount of success in inoculations with *Polyporus hispidus* on

ash trees. They have found the fungus growing in the tissues to some distance from the inoculated points.

Shaw (36) failed to get successful inoculations on sal trees, *Shorea robusta*, with *Polyporus shoreae* Wakef. He (35) reports successful inoculations by Hafiz Khan on Guazuma with *Fomes lucidus* (Leys.) Fr.

Gadd (25) states that inoculations with *Fomes lucidus* on two hevea trees have resulted in no ill effects.

Thompson (41) considers *Ganoderma lucidum* and *Fomes applanatus* to be only of subsidiary importance in bringing about infection on the oil palm, *Elaeis guineensis*, in Malaya, when considered in relation to *Fomes* sp., he isolated from diseased trees, which he thinks probably is related to *F. pachyphloeus*.

Nojima (28) has succeeded in inoculation experiments with *Polyporus japonicus* on oak roots in Japan.

De Jong (21) considers *Rigidoporus microporus* (*Fomes lignosus*) to be a weak parasite on rubber in the Dutch East Indies. Out of 21 trees he inoculated, 3 died within 2 years from inoculation.

The contrary view that in the suspected cases of parasitism of the Polyporaceae, *Rhizoctonia bataticola* is the organism concerned, has been advanced by Small (37) but has had little support from others.

The little success that has attended experiments in this direction is probably due to the slow-growing nature of the parasites. As White (48) says "it is difficult enough to make an inoculation without contamination, but it is much more difficult to prevent subsequent contamination during the months or even years through which the test must run, and at the same time to be sure of maintaining the moisture and air factors suited to the fungus. Moreover, we are very uncertain as to the extent of the counteracting influence exerted by the host in fresh wounds, such as inoculation incisions should be."

Accordingly, in the inoculation experiments to be described, although only pure cultures were used as inoculum, the conditions under which the inoculations were made were such as not to exclude contamination of foreign organisms. If checks are provided in sufficient numbers, they would be equally liable to this foreign infection; hence such scrupulous attention to sterile conditions is perhaps unnecessary.

The first inoculation experiments were made on young palms grown in the laboratory garden at Bangalore with the inoculum in the form of agar cultures and *Cassia siamea* root cultures. They were unsuccessful.

Further work was done on full-grown palms in the garden of the Government Experimental Farm at Babbur, a locality where the disease had not yet put in its appearance. Inoculum was prepared by growing the fungus on bits of *Cassia siamea* roots in Roux tubes with distilled water or

on soaked cotton in big test tubes $7'' \times 1''$. The fungus was allowed to grow for 2 to 3 weeks before inoculation, and was vigorously growing at the time. The inoculations were made by carefully digging the soil around the roots, cutting a root with a scalpel, placing the inoculum close to the wound, and covering up again with the soil. In some cases the inoculum was placed in sample tubes $2'' \times 1''$ into which the injured root was introduced; in a few others the inoculum was wrapped round the injured root in a piece of cellophane paper. Where the tree was inoculated in more than one root, other roots at different sides of the tree were chosen. There were check rows in between every 2 rows of inoculated palms. In a second experiment 3 rows of check trees were left in between two infected rows so as to prevent the possible growth of the roots of check trees towards the inoculum and thus getting the infection. Fifty-five palms were inoculated in all, of which 25 received 1 piece of inoculum each, 8 received 2 each, and 22 received 3 pieces each. Similarly, in the check rows, some trees were wounded in 1 root, some in 2, and some others in 3 roots, while there were also some unwounded checks.

In about 5 months one tree in a check row adjoining an infected row showed a yellowing of the crown, and in another 3 months the crown was dried up. At about this time one of the inoculated trees also showed the drying of the crown. The trees were then cut down, and the stumps brought to Bangalore. Some of the roots in both the palms were dry and discolored, as in the natural infection, and the characteristic mycelium with the clamp connections was noticed in the roots. The discoloration was also noticeable in the butt of the palm, and a microscopic examination showed the fungus in the discolored portion. Further examination was made in the field one year after the inoculation. Out of 11 palms examined, the roots of 5 showed fungous infection, those of 2 showed a slight discoloration and those of 4 were healthy. Of the last 4, the crowns of 2 palms showed yellowing. It was not thought advisable to disturb the roots of the other palms at that stage.

In the second experiment, at the end of 5 months the crowns of 3 trees were yellowish, and the basal leaves of 1 tree were showing signs of drooping. The roots in some cases were dry and brittle and the fungus was seen to be actively growing. Besides these one palm had the inoculum tied by wire to 2 roots near the surface of the ground, after removal of an outer slice of tissue by means of a scalpel. In this case, at the end of 6 days the fungus was growing well on the cut surface of the root. At the end of 5 months both these roots had been exposed by the irrigation water. The inoculum had disappeared from one root, but the root itself was dry and friable. Its base was discolored, the discoloration proceeding to the heart of

the palm. The tissues about 4 inches away from the inoculation showed the fungus. The other root had come away with the inoculum. It was dry and the end of the root still attached to the tree was rotten.

Four cocoanut palms in the laboratory garden were inoculated in the same manner as above with the cocoanut strain, but into the trunk bored with a round chisel. Within six months a slight bleeding was noticed in an inoculated tree (Fig. 1, C) but none in the check. The fungus was seen to be growing in the tissues in all 4 palms.

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SUMMARY

Ganoderma lucidum, a very common tropical fungus, causes a disease of areca and cocoanut palms in Mysore and other parts of India. The fungus has been brought into culture from the infected wood, and, on inoculating sterilized pieces of areca palm tissue, has produced sporophores with true pilei.

A study of some of the enzymes present in this fungus has shown that it secretes diastase, laccase, invertase, protease, coagulase, rennetase, and oxidase, and that it does not secrete maltase, lipase, and catalase. The decay it causes is of the brown-rot or "destruction" type; but, since it is capable of using tannin in cultures, it is probably a transitional form, like *Fomes annosus,* which disintegrates lignin and cellulose simultaneously.

The fungus grows best at pH 6.5, but is capable of growth between pH 3.0 to pH 7.0. On the alkaline side there is hardly any growth. The growth of the fungus tends to increase the initial acidity of the solutions.

Ganoderma lucidum appears to be a very slowly active parasite. Large pieces of inoculum cultivated on bits of *Cassia siamea* roots were introduced near living trees. The fungus was found to be growing in the roots and producing discoloration in 5 months. In one case of an artificially infected tree, death resulted and the fungus was found in the tissues. One of the control trees also died, probably due to its proximity to the infected trees; and, similarly, the fungus was found to have permeated its tissues.

An inoculation into cocoanut palms with the cocoanut strain resulted in inducing bleeding symptoms as in natural infection and the fungus was found to be growing in the tissues.

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THE SYMBIONTS OF PSEUDOCOCCUS BREVIPES IN RELATION TO A PHYTOTOXIC SECRETION OF THE INSECT[1]

WALTER CARTER

(Accepted for publication March 12, 1935)

INTRODUCTION

In a previous paper[2] attention was called to the fact that *Pseudococcus brevipes* (Ckl.) produced green spots on pineapple leaves at the points of feeding; and that this capacity was absent in mealy bugs from the roots of certain grasses but when present in the adult female mealy bug, was transmitted by her to her young. Since the symbionts of insects are similarly transmitted, the relationship of these organisms to the green-spotting capacity of the mealy bug has been studied with the object of checking the hypothesis previously set up[2] that an insect's symbionts conditioned its secretions. The fact that in this case the secretion is definitely phytotoxic adds significance to the results, since the majority of phytotoxic insects and virus carriers are sucking insects in which internal symbiosis is highly developed.

MATERIAL AND METHOD

Mealy bugs from heavily green-spotted pineapple plants were used. These were transferred in varying numbers and at various ages to panicum grass, *Panicum barbinode* Trin., growing in cloth-covered cages. After varying intervals of time, these mealy bugs or their progeny were returned to pineapple leaves for tests of their green-spotting capacity. Concurrent with these tests, examinations were made from time to time of the symbiont flora of individuals from the colonies of mealy bugs used. The method here was to dissect out the insect's mycetome, smear it rapidly on a slide, and, after a short period of air drying, stain with Heidenhain's iron-haematoxylin. Some 500 preparations were examined during the course of this study, and mealy bug colonies have been kept under observation for upwards of 2 years.

EFFECT OF THE HOST PLANT TRANSFER

A. On green spotting. When mealy bug colonies were allowed to feed for short periods of 10 to 14 days on panicum grass and then returned to pineapple leaves, the latter became green-spotted. Such experiments indicated that length of feeding time on the grass was a factor.

[1] Published with the approval of the Director as Technical Paper No. 81 of the Experiment Station of the Pineapple Producers Cooperative Association, University of Hawaii.

[2] Carter, Walter. The spotting of pineapple leaves caused by *Pseudococcus brevipes*, the pineapple mealy bug. Phytopath., 23: 243–259. 1933.

The method of using gravid females was then employed. Numbers of gravid females were introduced into the cages containing grass in vials covered with cloth in such a manner that only the new-born crawlers could reach the grass. As checks on the green-spotting capacity of the colony, additional females were transferred to pineapple leaves. The colonies produced by these check females invariably produced spots.

A large number of small cages of grass, each containing 25 females, were set up in this manner. The numbers of young mealy bugs establishing themselves on the grass were very small. One effect of the host transfer was the immediate production of a large percentage of males that were useless for further study; another was to slow down the development of the insect so that from many of these cages only 3 or 4 specimens were obtained. The method was then used of transferring hundreds of gravid females direct to the stems of panicum in larger cages. From these it was possible to obtain an adequate supply of material.

In table 1 are summarized the data from 8 series of tests with the progeny of gravid females removed from green-spotting colonies to panicum grass. In 7 of these tests the green-spotting capacity survived in some individuals.

TABLE 1.—*Transfer of gravid female mealy bugs from pineapple to panicum grass. Summary of tests with first generation*

Date females transferred to panicum	Date progeny transferred to pineapple	Green spotting by progeny		Dissection of progeny	Condition of symbionts
		+	−		
11/15/33	1/1/34	1	2	12/14/33	Rod-shape form
				1/22/34	"
11/21/33	.....	...	...	12/7/33	"
11/15/33	1/10/34	3	0	12/8/33	"
				1/10/34	"
11/29/33	1/10/34	2	0	No record	No record
11/29/33	1/22/34	2	0	No record	No record
2/28/34	5/1/34	4	2	5/21/34	Rod-shape form
2/28/34	4/6/34	5	51	4/6/34	Coccus rods
				5/15/34	Rod-shape and intermediate forms[a]
2/20/34	3/20/34	0	100	3/20/34	Intermediate in 1; coccus rod in 9

[a] 5/15/34 dissection was of green spotting individuals.

Evidence that green spotting in first-generation mealy bugs on panicum is limited to those individuals and not transmitted to their progeny is seen in figure 1 (left), which represents graphically the history of a colony car-

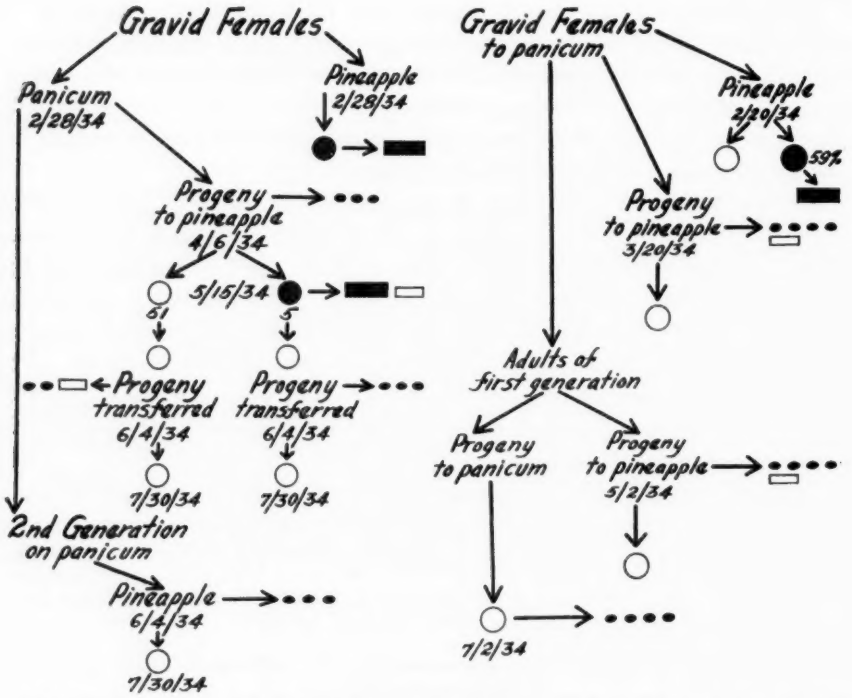

FIG. 1. Colony histories showing results of green spotting tests and the condition of the rod-shape symbiont. *Legend:* Black circle, green spots; hollow circle, no green spots; black bar, typical rod-shape form; hollow bar, intermediate form; oval, coccus rod.

ried to second generation on panicum grass. None of the second generation produced spots when returned to pineapple leaves.

Figure 1 (right) is a diagram of a colony history in which the green-spotting capacity was lost in the first generation. This was, no doubt, related to the fact that only 59 per cent of the mealy bugs in this colony were producing green spots when transferred to panicum, so that the colony as a whole responded more rapidly to the changed environment.

Continued growth of all these colonies on pineapple plants has failed to restore the green-spotting capacity.

THE SYMBIONTS OF PSEUDOCOCCUS BREVIPES

These are described more fully in a concurrent paper.[3] Since this study concerns only the rod-shape symbiont and its forms, no reference is made herein to the so-called "common symbiont." Previous data[4] on the pres-

[3] Carter, Walter. The symbionts of *Pseudococcus brevipes* (Ckl.). Ann. Entom. Soc. Amer. 28: 60–64. 1935.

[4] See footnote 2.

ence of the organism in the mycetome of green-spotting mealy bugs have been fully confirmed by these later studies. Dissections of such mealy bugs have, without exception, shown the organism present in large numbers (Fig. 2, A). When a long series of mealy bugs from a green-spotting mealy-bug colony is taken and dissected without first individually testing for green spotting, the symbiont is found in varying numbers in different individuals, in some cases being recordable as present only in very small numbers. Colonies of the type listed in figure 2 show this variation in numbers of the

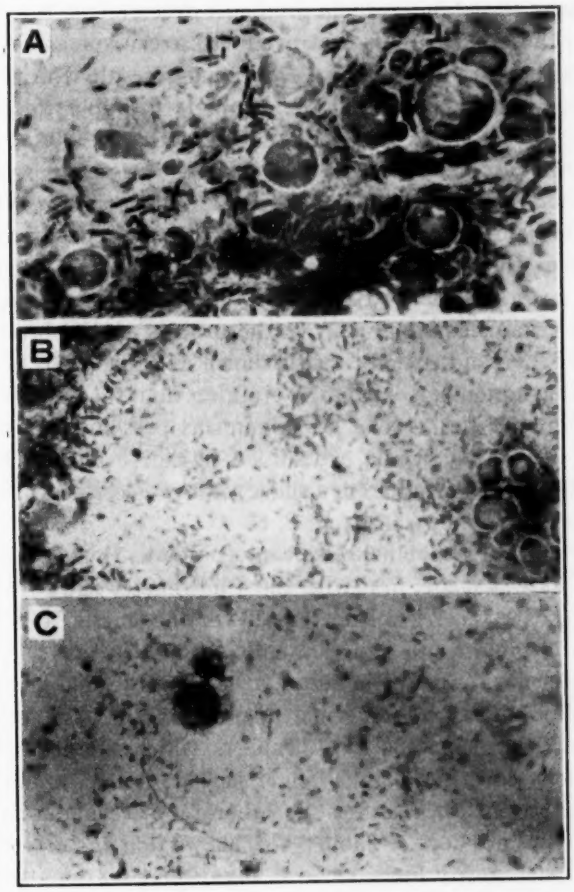

FIG. 2. The rod-shape symbiont of *Pseudococcus brevipes* (Ckl.) and its pleomorphic forms. A. Typical rod from green spotting mealy bug. B. Intermediate form. C. Typical coccus rod from non-green spotting mealy bug.

symbiont to a pronounced degree. The evidence of such series establishes the fact that the symbiont "culture" in the mycetome of the insect shows fluctuations in numbers common to other active populations of organisms.

EFFECT OF THE HOST PLANT TRANSFER

B. On the rod-shape symbiont. When green-spotting mealy bugs are transferred from pineapple to panicum grass, the organism disappears. This disappearance is coincident with the loss by the mealy bug of the capacity to produce green spots. In table 1 the presence or absence of the organism is shown. It is seen from this table that as long as progeny produced spots after being removed from the panicum grass to pineapple leaves, the symbiont could be found. When larger populations were used and only a small percentage of the individuals produced green spots, the organism was found only in those individuals that produced green spots. When transfer of mealy bugs back to pineapple leaves no longer resulted in the production of green spots, dissections showed the complete disappearance of the symbiont.

The optical equipment used to determine the presence of the organism was a 4-millimeter objective and a × 5 ocular. With practice, a 16-millimeter objective could be used for preliminary viewing of preparations. An occasional case where the symbiont appeared to be smaller than normal led to the use of higher magnifications. It was then found that these occasional smears contained the organisms apparently in the process of dissolution. The best marked case of this kind encountered is shown in figure 2, B. In this preparation, normal symbionts, more faintly staining than the other forms present, were found, but the majority of the organisms present were narrower and shorter than the normal, while, with a 1.4 objective and a ×10 ocular, extremely small bodies varying from a short rod to a coccus form could be seen. These last-named are shown in figure 2, C.

Evidence that the symbiont was pleomorphic was thus obtained and from that time search was made, first with the 4 millimeter ×10 for the rod-shape form and then with the 1.4 millimeter ×10 for the smaller form.

Reference to table 1 and figures 1 and 2 will show that the transition or intermediate form is found in rare cases in first- and second-generation mealy bugs on panicum, but that the normal form for mealy bugs from non-green-spotting colonies is the coccus rod. Table 2, which is a colony history of a mealy bug colony covering a much longer period of time, shows that the coccus rod is the stable form under the laboratory-greenhouse conditions in which the colonies are kept. Table 2 also shows that the coccus-rod populations vary in such mealy bugs, since in many preparations they are found only with great difficulty. The intermediate form is closer in form to the coccus-rod than to the rod-shape form, and occasionally preparations are found that contain scattered individuals in a coccus-rod population that are larger than the average coccus rod. Such cases are recorded as intermediates, though exact criteria for determination are not available. The 3 cases recorded in table 2, under date of January 5, 1934, are cases in point.

TABLE 2.—*Green spotting tests and dissections of mealy bugs after a green spotting colony had been transferred to panicum grass and had developed thereon from January 5, 1933, to August 12, 1933*

Dates	Green-spotting tests	Dissections				
		No.	Symbionts			
			Rod	Intermediate	Coccus rod	Absent
8/12/33	Negative	21	0	0	10	11
9/16/33	"	0				
10/31/33	"	0				
12/5/33	"	0				
1/5/34	"	14	0	3[a]	14	0
2/13/34	"	0				
5/1/34	"	10	0	0	10	0

[a] Determination based on a few scattered individuals in coccus-rod population that appeared slightly larger than average.

DISCUSSION

The symbionts of *Pseudococcus brevipes* are host-bound and are not themselves transmitted by the insect's feeding.[2] The relationship between the rod-shape symbiont and green spotting must therefore be brought about by the influence of the former on the oral secretions of the insect. In the case of *P. brevipes,* the mycetome is invariably found in close apposition with the large mid-gut of the insect, although no connecting duct between the two has been observed.

The factor of time involved in the complete disappearance of the symbiont from the insect's mycetome suggests that the critical time is when the transfer of the symbionts takes place from the mother to the young. Indications of this are the persistence of green spotting in mealy bugs that have fed on panicum for short periods of time only (10–14 days), the partial disappearance when the mealy bugs are first applied to panicum as unfed crawlers, and the complete disappearance of the organism in mealy bugs whose parents have lived their entire lives on panicum.

Without recognition of the transitional or intermediate form of the symbiont, the relationship between the rod-shape form and the coccus-rod form could not have been suspected. In addition to the cases tabulated, the intermediate form together with sparse numbers of the rod-shape form, has been found in individuals from a green-spotting colony which were temporarily unable to produce green spots. This suggests that fluctuations in the rod-shape symbiont population in individuals of a green spotting colony are responsible for the proportion of these individuals which at any one time are unable to produce green spots on the first leaf to which they are transferred.

With the normal development of the colony, such individuals or their progeny become capable of producing green spots through the development of the rod-shape population in the insect. When, however, the food plant of the insect is radically changed, as it is when panicum grass is substituted for pineapple, the life cycle changes and, instead of the organism merely fluctuating in numbers, or from rod to intermediate stage and back to rod, there is a degeneration to the minute coccus-rod form. Populations of this last form are always relatively low compared with those of the rod and frequently are so small as to make determination difficult.

The field situation over most of the pineapple-growing area of Hawaii is one wherein the green-spotting capacity, in greater or less degree, is always present and, under natural conditions, it is evident that the stability of the coccus-rod condition is broken in some way and the rod-shape symbiont, with its concomitant green-spotting capacity, restored. In the laboratory-greenhouse conditions, wherein all the colonies are strictly isolated, this has not yet taken place, even though mealy bug colonies from which the green-spotting capacity has been eliminated by feeding on panicum grass, have been fed on pineapple plants for long periods of time.

While the restoration of the rod-shape form and the green-spotting capacity would be a significant completion of a chain of evidence, especially if the stimulus could be determined, the facts indicate that the mere change of food was not responsible for the loss of the mealy bug's capacity to produce green spots. If the latter were true, then the colony should promptly resume its green spotting when returned to pineapple. On the other hand, the invariable presence of the rod-shape form in green-spotting mealy bugs, the evidence of a transitional form, the complete absence of the rod and the invariable presence of the coccus-rod in non-green spotting mealy bugs are considered to be proof of the relationship between the symbionts and oral secretions of the insect.

SUMMARY

The relationship between the symbionts of *Pseudococcus brevipes* (Ckl.) and the phytotoxic secretion of the insect that is responsible for green spotting of pineapple leaves has been studied. Green-spotting mealy-bug colonies were transferred from pineapple to *Panicum barbinode* Trin. and transferred back to pineapple after varying lengths of time.

The effect of the colonies' feeding on the grass was to eliminate the green-spotting capacity of the mealy bugs; continued growing of these colonies on pineapple has failed to restore this capacity.

Coincident with the loss of the green-spotting capacity is the disappearance of the rod-like symbiont from the mycetome of the insect. The evidence is that this symbiont is pleomorphic and passes from a rod-shape to a coccus-rod form under the influence of radically changed nutrition.

The rod-shape form is invariably present in green-spotting mealy bugs; the coccus-rod in non-green spotting mealy bugs. The fact that return of the mealy bugs to pineapple does not result in the return of the capacity to produce green spots is proof that the loss of this capacity in the first instance is not due merely to a changed food plant, but to a radically changed physiology that is clearly associated with pleomorphism of the insect's symbionts.

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SCLEROTINIA ROT OF SQUASH AND PUMPKIN[1]

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(Accepted for publication March 21, 1935)

Many rotting spots developed in squash and pumpkin fruits in gardens and in storage in Bozeman, Montana, in 1933 and 1934. These fruits were studied to determine the cause of the disease.

REVIEW OF LITERATURE

Young and Morris (23) and Young (22) described the sclerotinia stem canker of sunflower and hollyhock. Joshi (10) described sclerotinia wilt of safflower, and reported that artificial inoculations with this fungus caused infections in wheat, oats, and gram. The correct name of the causal fungus is *Sclerotinia sclerotiorum* (Lib.) deBary, as determined by Brooks (4). Young (21) gave a preliminary description of sclerotinia rot of pumpkin and squash.

This sclerotinia continues to attack an increasingly large number of individuals and species of economic plants, and to infest increasing amounts of the best agricultural soil. This fungus does its worst economic damage to cultivated species mostly in the following families: *Compositae, Cruciferae, Cucurbitaceae, Chenopodiaceae, Leguminosae, Solanaceae*, and *Umbelliferae*. It probably will become more destructive in the countries with temperate climates. Consequently, the following list of hosts with citations is given for the convenience of the many people who study and work to control this extremely destructive fungus.

Plants diseased by *Sclerotinia sclerotiorum*

Aegopodium (14)
Alfalfa (2, 3)
Amaranthus[a] (23)
Angelica (15)
Antirrhinum (3, 7)
Apple (3)
Apricot (16)
Aquilegia (1)
Argemone (10)
Artichoke (4, 18)
Asphodelus[c] (10)
Bean[a] (6, 19, 23)
Beet[a] (6, 18, 23)
Brassica[a] (3, 15, 23)
Cabbage (3, 6, 18)
Campanula (7, 23)
Canada thistle[a] (3, 23)
Cantaloupe (1)
Caraway (6)
Cardamine (3)
Carrot[a] (3, 6, 18)
Cauliflower (6, 18)
Celery[a] (6)
Cerefolium (14)
Cheiranthus (3, 7)
Chenopodium[a] (23)
Chicory (6, 18)
China aster (15)
Chrysanthemum[a,b]
Clover (2, 18)
Cnicus (3)
Columbine (17)
Cucumber (6, 19)
Dahlia (17, 23)
Delphinium (3, 15)
Easter lily (1)
Eggplant (6)
Fanweed[a] (23)
Fennel (18)
Fig (20)
Flax (12)
Foeniculum (14)

[1] Contribution from Montana State College, Agricultural Experiment Station, Paper 51, Journal Series.

Forsythia (15, 18)
French endive[a] (23)
Ginseng (17)
Gram[c] (10)
Hemp[a] (15, 18)
Hollyhock[a] (22)
Ipomoea (15)
Iris (1)
Iva[a] (23)
Jerusalem artichoke (9)
Kale (6)
Kohlrabi (6)
Lamium (18)
Lathyrus (19)
Lavatera (3)
Lemon (3, 18)
Lettuce[a] (6, 18)
Lupinus (1, 3)
Mangel-wurzel (6)
Melilotus[a, b]
Mulberry (5, 18)
Mustard (3, 18)
Myosotis (11)
Oat[c] (10)
Omphalodes (18)
Onion (6)
Parsley (6)
Parsnip (2, 3)
Passiflora (3)
Pea[a] (3, 6, 23)
Pear (3)
Pepper (6)
Petunia (14)
Potato[a] (3, 6, 23)
Pumpkin[a] (3, 21)
Radish (14, 18)
Rape (3, 18)
Rhubarb (6)
Rock melon (3)
Rutabaga[a] (6, 23)
Safflower (10)
Salsify (15)
Schizanthus (7)
Silybum (3)
Sonchus (3, 23)
Sour orange (3, 15)
Soybean (2, 3)
Spinach (6)
Squash[a] (21)
Strawberry (1)
Sugar beet[a, c] (23)
Sunflower[a] (23)
Swede (3)
Tobacco (1, 3)
Tomato (3, 6, 18)
Trigonella (13)
Turnip (3, 6)
Vicia (3, 15, 18)
Violet (19)
Watermelon (1, 3)
Wheat[c] (10)
Zinnia[a] (3, 14, 18)

[a] In Montana.
[b] Described in this article.
[c] Artificial inoculation.

MATERIALS AND METHODS

Rotting fruits of pumpkin and squash were brought to the laboratory and a fungus from them was isolated on agar. The agar cultures were inoculated into shallow wounds in healthy fruits of squash and pumpkin to determine under laboratory conditions the symptoms of infection. The fungus was reisolated from the artificially infected fruits.

Sclerotia produced in naturally infected pumpkins and squashes were planted in sand in flower pots in the greenhouse. The sand was covered with cloth and kept wet during a few months to facilitate production of apothecia by the sclerotia. Henson (8), Joshi (10) and Young (22) produced apothecia from sclerotia on agar.

EXPERIMENTAL RESULTS

In the pumpkins (*Cucurbita pepo* L.), the mold caused a rapidly developing soft rot that released a large amount of liquid, and produced dense masses of white mycelium with numerous black sclerotia ranging in size from 0.5 to 15.5 cm. long (Fig. 1, A, D). Large masses of mycelium grew from the sides of pumpkins set with their infected spots over jars (Fig. 1, C).

The mold caused disc-shape, rapidly enlarging, water-soaked spots 1 to 10 cm. wide in the squash (*Cucurbita maxima* Dach.) fruits (Fig. 1, B). These spots developed a dry rot that changed Buttercup and Table Queen

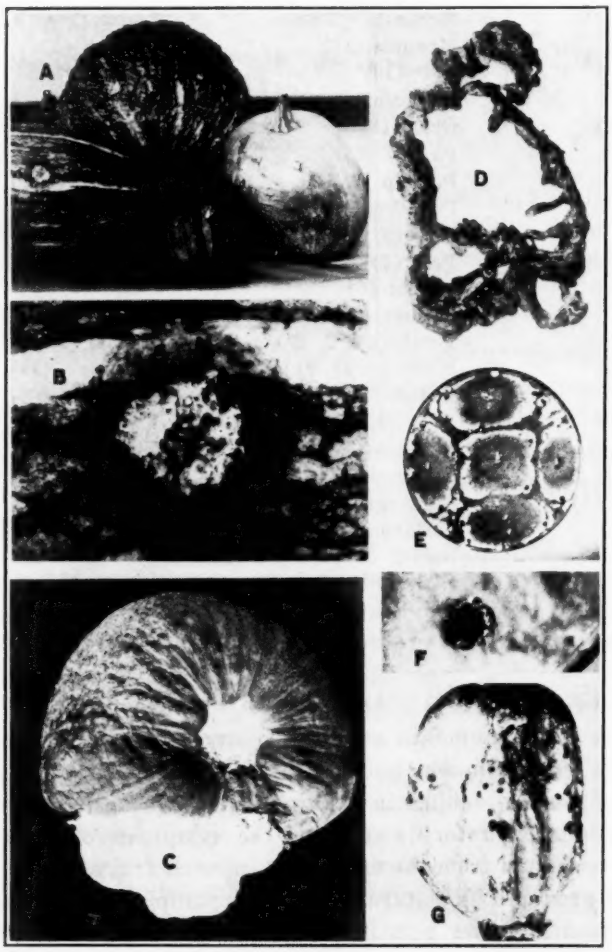

FIG. 1. *Sclerotinia sclerotiorum.* A. Epidermal wrinkles in a Winter Luxury pumpkin, and fruit spots with white mycelium in Winnebago, Warrens Improved, and Table Queen squashes, as symptoms of natural field infection with Sclerotinia. The infected parts did not touch the soil. The globular Warrens Improved squash weighed 21 lb. B. Large water-soaked ring surrounding Sclerotinia hyphae in a Winnebago squash. This ring marked tissues killed by the fungus. C. Mass of Sclerotinia mycelium produced on a Small Sugar pumpkin. ×4/17. D. A sclerotium of Sclerotinia produced inside a naturally infected Small Sugar pumpkin. ×½. E. Antagonism for each other shown by 5 separate colonies of *S. sclerotiorum* in a pure culture on an agar plate. This fungus was reisolated from an artificially inoculated Buttercup squash. ×¼. F. Juvenile sclerotium with exuded liquid droplets. *Sclerotinia* isolated on agar from a Winter Luxury pumpkin. ×1. G. Artificially inoculated Table Queen squash bearing sclerotia and mycelium of Sclerotinia. Many of the sclerotia were like that in F. Squashes rotting in storage bore almost no external mycelium.

squashes into dry mummies containing many sclerotia. The seeds were decomposed in the rotted squash and pumpkin fruits.

In 1933, most of the squash and pumpkin fruits were infected in spots that did not touch the soil. The infections appeared as small, water-soaked spots with white mycelium protruding from their centers (Fig. 1, A, B). Droplets of yellow liquid were exuded from the margins of many of the infected spots. The infection of these fruits was associated with 2.72 inches of rain from Aug. 21 to 27, 1933. These fruits exude sap from wounds, and this sap would serve as a culture medium for the saprophytic development of ascospores of Sclerotinia preceding infection. Hence, the infection of the tops of these fruits may have been caused by ascospores of Sclerotinia discharged during the rainy period. During 1934, the fungus entered the bottoms of the fruits touching the soil, so mycelium in the soil presumably caused these infections.

Isolations from the rotting pumpkins and squashes yielded many pure cultures of *Sclerotinia sclerotiorum*. The mycelium on both agar and fruits aggregated to form dense masses that exuded many droplets of liquid, while they produced black, hard sclerotia (Fig. 1, D, F).

The Sclerotinia on agar was placed in wounds in 12 healthy fruits of pumpkin and squash in the laboratory. Ten of the fruits developed typical sclerotinia rot from the wound inoculations (Fig. 1, G). The 27 fragments of host tissues taken from these fruits and placed on agar all made pure cultures of *Sclerotinia sclerotiorum* (Fig. 1, E).

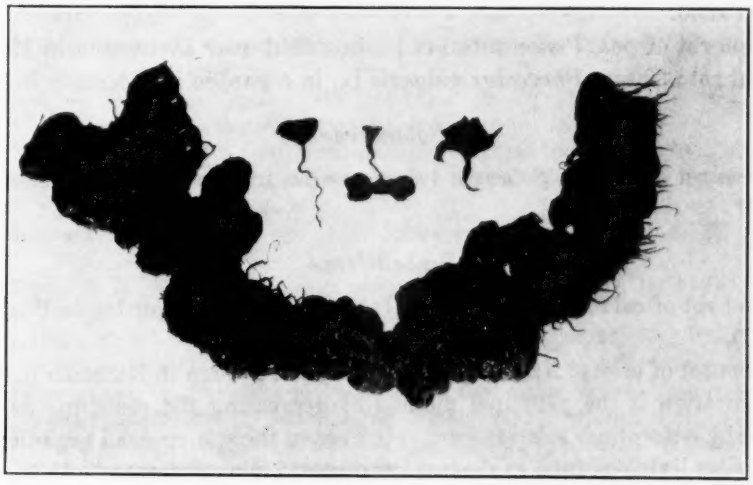

FIG. 2. Apothecia of *Sclerotinia sclerotiorum* produced by sclerotia planted in sand. These sclerotia had grown in pumpkins. The central mature apothecium is attached to its sclerotium. The large sclerotium bore only immature apothecia. ×1.

During the first 2 weeks of growth, none of the 5 main colonies coalesced in one of these plates of *Sclerotinia sclerotiorum* (Fig. 1, E). This antagonism between the colonies was less definite than the antagonism previously observed between colonies of other fungi and bacteria.

Many sclerotia from naturally infected squash and pumpkin fruits were planted in sand in the greenhouse. The sclerotia from squash did not produce apothecia. However, the sclerotia from two pumpkins produced 17 typical apothecia and many filiform, atypic apothecia within 128 to 208 days (Fig. 2).

Besides the hosts previously reported by Young and Morris (23) and Young (21), *Sclerotinia sclerotiorum* naturally infected and caused serious diseases in the following economic plants in Montana.

Compositae

Root and stem rot of shasta daisy, *Chrysanthemum maximum* Ram., at Ronan in 1933.

Drop of head lettuce, *Lactuca sativa* L., in a field near Bozeman in 1929.

Stem rot of *Zinnia elegans* Jacq. in a garden in Bozeman in 1934.

Leguminosae

Stem rot of yellow sweet clover, *Melilotus officinalis* Lam., in a greenhouse at Bozeman in 1932.

Stem rot of white sweet clover, *Melilotus alba* Desr., in a field at Bozeman in 1930.

Stem rot of pea, *Pisum sativum* L., in a field near Livingston in 1932.

Pod rot of bean, *Phaseolus vulgaris* L., in a garden in Bozeman in 1933.

Solanaceae

Stem rot of potato, *Solanum tuberosum* L., in a greenhouse in Bozeman in 1931.

Umbelliferae

Root rot of carrot, *Daucus carota* L., *sativa* DC., in a garden in Bozeman in 1929.

Stem rot of celery, *Apium graveolens* L., in a garden in Bozeman in 1930.

Sanitation is the principal method of decreasing the economic damage caused by *Sclerotinia sclerotiorum*. It lives in the soil on dead organic matter besides living plants, so destroying diseased plants decreases the spread of the fungus in the soil. Soil sterilization controls sclerotinia in greenhouses. Rotation of crops and the use of resistant plants are practical methods of control.

SUMMARY

Sclerotinia sclerotiorum caused a wet rot of pumpkin fruits and a dry rot of squash fruits, producing abundant white mycelium and many large black sclerotia in the fruits. In pumpkin, it produced sclerotia 0.5 to 15.5 cm. long. These sclerotia produced apothecia when planted in sand.

This Sclerotinia was isolated from naturally infected squashes and pumpkins; was inoculated into healthy squashes and pumpkins in which it caused typical rot; and was reisolated from these artificially inoculated and rotting fruits.

The following are reported as new hosts of this Sclerotinia in Montana: Bean, carrot, celery, lettuce, pea, potato, shasta daisy, white sweet clover, yellow sweet clover, and zinnia.

The known hosts of *Sclerotinia sclerotiorum* are listed, with citations.

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INOCULATION OF RABBITS WITH ELSINOE AMPELINA

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(Accepted for publication February 2, 1935)

Charrin and Le Play's report of the infection of animals (only rabbits are named) with a culture of *Elsinoe ampelina* (de Bary) Shear,[1] or what they supposed to be this fungus, was published by Viala and Pacottet (7, p. 521–523) in connection with their discussion of anthracnose of grape (*Vitis* sp.), which is caused by this organism. The writers' recent inoculations of rabbits with an authentic culture of this plant pathogen gave only negative results. These experiments are here described and certain other sustaining data presented, particularly because they explain to some extent Charrin and Le Play's results as interpreted by them, thus serving as a correction of the literature as it stands at present.

In Charrin and Le Play's report of their inoculations, supposedly with *Elsinoe ampelina,* reference is made to their previous infection (3) of animals with another fungus from grape, *Stearophora radicola* Mangin and Viala (4). Severe infection was obtained with both rabbits (3, 4) and guinea pigs (4). *Oospora guignardi* also is mentioned by Charrin and Le Play (7, p. 523) as associated with lesions of a rabbit; it is believed, however, that the intended reference is to an undetermined species of Oospora isolated by Charrin and Delmare (2) from a cystic tumor of a rabbit and identified (2) as closely related to this grape fungus. The cultures of the Stearophora were provided by Viala and Pacottet and the inoculations were made by means of subcutaneous and intraperitoneal injections of the fungus (3). It is inferred that the cultures, supposedly of *E. ampelina,* were obtained also from Viala and Pacottet and that the inoculations with it were made by means of intraperitoneal, if not also subcutaneous, injections of the fungus.

In each of the 2 inoculation experiments performed by the writers 3 groups of rabbits were inoculated. Of these groups, consisting of 2 animals each, the first received subcutaneous inoculation, the second, intraperitoneal, and the third, intravenous. The culture of Elsinoe employed as inoculum was isolated in October, 1924, from anthracnose lesions on grape from Florida, and it had produced severe infection when inoculated on grape.[2] Cultures grown on potato-dextrose agar slants were employed as in the case of the inoculation experiments on the plant suscept. In the first experiment the cultures were 3 months old and in the second, 1 month old (Fig. 1, A).

[1] As discussed by Shear (5), this fungus originally described as *Sphaceloma ampelinum* de Bary (1) was assigned the name *Manginia ampelina* by Viala and Pacottet (6, p. 145–150).

[2] Unpublished data.

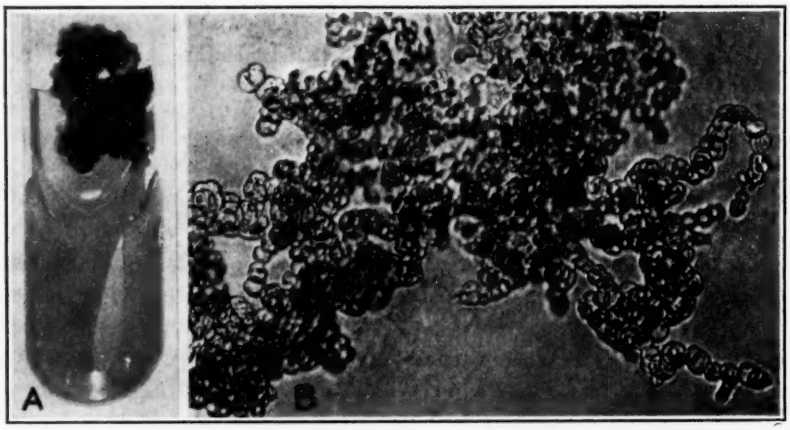

FIG. 1. *Elsinoe ampelina.* A. Month-old growth of the fungus on potato-dextrose agar. B. Young hyphae from a plating on beef agar. ×260.

In each case the purity and vitality of the cultures were determined by making platings from them on beef agar several days before the inoculations were to be made. Hyphal growth composing young cultures from a plating is shown in figure 1, B. By platings similar to those just described, it was demonstrated also that the growth of the organism was not inhibited by the saline solution.

Although they may have been present in some instances, no conidia were found in the mounts made from the potato-dextrose agar cultures actually used for inoculating grape, or rabbit, nor in the beef-agar cultures. They form readily, however, when cultural growth is transferred to a moist atmosphere; if conidia are necessary for infection of grape, in the inoculation experiments performed they must have formed soon after the water infusion of the culture was applied to the plant.

That the results of the inoculation experiments on rabbits would be negative was rather to be expected, if for no other reason than that the Elsinoe would probably not be viable at its body temperature, *i.e.*, 38°–39° C. In lieu of information on the exact thermal death point of the fungus, cultures were incubated at 37½° C., which is slightly lower than the body temperature of the rabbit.

The first inoculation experiment was performed on June 27. One of the subcutaneously inoculated animals became injured in the cage on July 20, later developing posterior paralysis. It died on August 6. The autopsy revealed a small local abscess at the site of inoculation, but no gross lesions attributable to the Elsinoe were found. The 5 remaining animals included in the experiment were autopsied on August 7 and 9. On the other subcutaneously inoculated animal there was also a small abscess at the site of

inoculation. A small pedunculated nodule containing dry, yellowish pus and probably of parasitic origin was found in the peritoneum of one of the animals inoculated intraperitoneally. No gross lesions attributable to the inoculated organism were present, however, and the animals were well nourished.

In the second inoculation experiment, performed on August 3, one of the animals died on September 12. The autopsy here revealed the presence of an enteritis, which was probably largely responsible for the death of the rabbit. No lesions attributable to the Elsinoe were found. The other 5 animals included in this experiment were autopsied on October 15 and 16. Small local abscesses were again present at the site of inoculation of the 2 animals subcutaneously inoculated; otherwise, no lesions attributable to the Elsinoe were found. The animals were in a state of good nutrition.

It is believed that in both experiments the local abscesses resulted merely from the introduction of a foreign substance under the skin.

At the time of autopsy cultures were made from the heart's blood, and internal organs, lungs, liver, kidneys and spleen, of all of the inoculated animals, with the exception of the one that died on August 6, whose internal organs were not suitable for culturing; the four local abscesses and the peritoneal lesion also were cultured. The media used were potato-dextrose, glycerin, and Sabouraud's maltose agar, all of which are favorable for the growth of the Elsinoe. In no case was the fungus isolated and for the most part the cultures remained sterile. Some developed *Bacillus coli* and a few showed fungus growths.

The several sets of cultures incubated at 37.5° C. for approximately 10 days ceased their development at this temperature and failed to grow when removed to room temperature, or when transferred to fresh media. The fact that the fungus was killed at 37.5° C. not only substantiates the negative results of the inoculation experiments here reported, but it also precludes the possibility of the infection of rabbits by this fungus as reported by Charrin and Le Play.

A partial solution, at least, of the positive results reported by Charrin and Le Play, *i.e.*, if the lesions they describe were caused by the inoculum they used, is that this was entirely of some other fungus, or was impure for the Elsinoe. If their cultures were obtained from Viala and Pacottet, as has already been suggested, they may well have been mostly or entirely of some other fungus. In support of this statement may be cited Viala and Pacottet's assertion that the fungus they studied as *Elsinoe ampelina* was similar to *Colletotrichum lindemuthianum* in general cultural appearance (8, p. 375) and that it was of rapid growth in culture (6, p. 120–121). Neither characteristic applies to the Elsinoe. This fungus grows so slowly in culture that a faster growing organism associated with it on anthracnose lesions

could have been isolated much more readily than the Elsinoe, and then have been confused with it. On other grounds Shear (5) has previously questioned the purity of Viala and Pacottet's cultures, supposedly of *E. ampelina*.

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AUSTRALIAN CITRUS SCAB CAUSED BY SPHACELOMA FAWCETTII SCABIOSA

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(Accepted for publication May 29, 1935)

The "yellow rough scab" (1) of lemon leaves, said to have been common in Queensland in 1876, evidently is the same as the "orange leaf scab" that Tryon (1) reported from Toowoomba, Queensland, in 1889, attributing it to *Ramularia* sp. Ten years later McAlpine (2) designated it as *R. scabiosa* McAlpine and Tryon. He used the term "scabbing" of citrus in a collective sense; and, referring to the Queensland disease reported in 1876, suggested that it might be traced to "some of the native Citrus trees." Florida citrus scab or verrucosis, first attributed to *Cladosporium* sp., now identified as *Sphaceloma fawcettii* Jenkins, he said had not yet been detected in Australia.

At the request of E. J. Butler, 2 specimens of scab collected by Tryon at Toowoomba, and doubtless sent by him to McAlpine and retained at the Department of Agriculture, Melbourne, Victoria, were transmitted to the writer by D. B. Adam (Dec. 3, 1926). Both are labelled in what is probably McAlpine's handwriting, one (Fig. 1, G) as *Ramularia scabiosa,* the other (Fig. 1, H and I), described as occurring on lesions with the Ramularia, as *Phyllosticta scabiosa* McAlpine. The 2 specimens, both showing the Ramularia, are then of authentic value, and are probably to be classed as type specimens. The Ramularia on this material is certainly the same as the fungus on a similar specimen of what is now called scab in New South Wales, kindly contributed (May 12, 1926) by G. P. Darnell-Smith. As the author wrote to Dr. Darnell-Smith (Aug. 30, 1926), this is a Sphaceloma similar to *S. fawcettii,* but sufficiently distinct to suggest a different species. The conidia (often 10–17 $\mu \times 2.5$–5 μ) and conidiophores are noticeably larger than in *S. fawcettii.* The lesions are also larger, as well as more regularly discoid or crateriform than those of the Florida citrus scab, and the more robust fructifications are more easily seen (Fig. 1, A–F). These are grayish, hair brown or fuscous (3). Fructifications of *S. fawcettii* are ordinarily more delicate in appearance and generally drab, although they may become dark. The difference in the lesions may be seen by comparing illustrations of the 2 diseases found in the literature (4, 5). For the present time at least these considerations seem to necessitate making a new name for *R. scabiosa,* and for this *Sphaceloma fawcettii scabiosa* is proposed.

McAlpine reported citrus scab caused by the form of *Ramularia scabiosa* on leaves only, although Tryon also recorded it on fruit. The fruit scabbing that McAlpine attributed to several new species of imperfect fungi, including *Cladosporium subfusoideum,* also recorded on leaves, is evi-

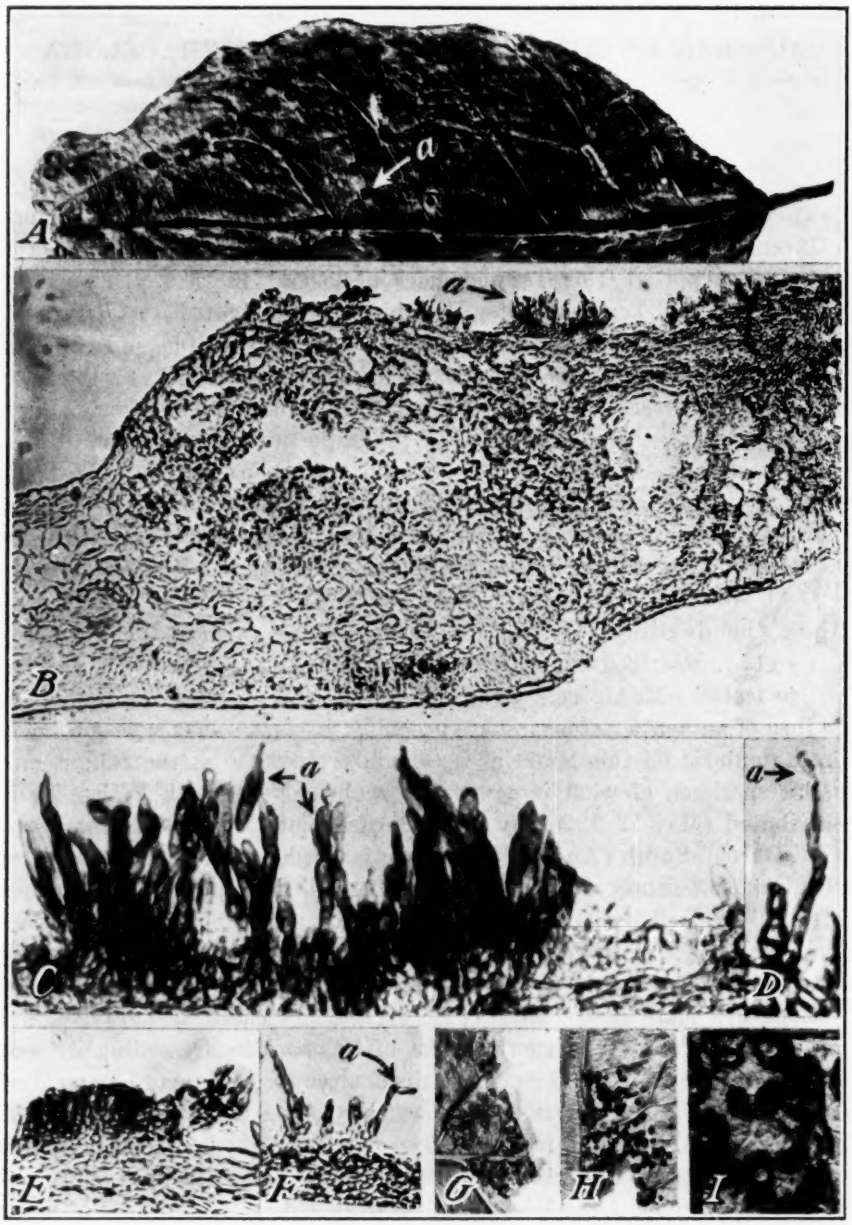

FIG. 1. A–F. *Sphaceloma fawcettii scabiosa* on lemon leaf from G. P. Darnell-Smith, N. S. Wales. A. Portion of leaf. ×1. B. Section of leaf through lesion at A, *a*. ×100. C, D. Conidiophores bearing conidia (C, *a* and D, *a*), those in C enlarged from B, *a*. ×600. E. Acervulus at edge of lesion. ×400. F. Additional conidiophores and a conidium (F, *a*). ×400. G–I. *Ramularia scabiosa*, original collections by Tryon. G. Portion of orange leaf. ×1. H. Portion of lemon leaf. ×1. I. Portion of H, enlarged. ×5.

dently the same disease. The late N. A. Cobb, who worked many years in Australia, agreed with the writer that the grey scab of lemon he reported (6) in New South Wales was apparently Australian citrus scab. It is probable that the scab of citrus first reported in New Zealand by Kirk (7), as well as the disease known in that country as grey scab (4) of citrus, may be the same disease.

The early Queensland scab record (1876) constitutes the first definite report of a disease of citrus caused by *Sphaceloma* in any country, as well as one of the first *Sphaceloma* diseases ever reported. Further field and herbarium taxonomic study should be made to determine whether *Sphaceloma fawcettii scabiosa* should properly be given specific rank and whether, as suggested by McAlpine, it is perhaps indigenous to the Australian continent on native citrus.

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PHYTOPATHOLOGICAL NOTE

Soil Nematodes in Forest Nurseries.—During the last 2 years there have been observed in Wisconsin forest nurseries several cases of a direct correlation between the severity of damping off of coniferous seedlings and the number of microscopic eel worms, or nematodes[1] occurring in soil. It was particularly striking that the parasitic organisms in the soils heavily infested with nematodes were highly resistant to the common methods of damping-off control. In all cases the soils had been previously treated with farm manure. All of the nematodes observed possessed the same common characteristics; they were smaller than 0.3 mm. in length and .02 mm. in width, with a cylindrical, elongated, transparent, nonsegmented body, pointed at both ends (Fig. 1). They compared closely with the genus Rhabditis, but no attempt was made to identify the species.

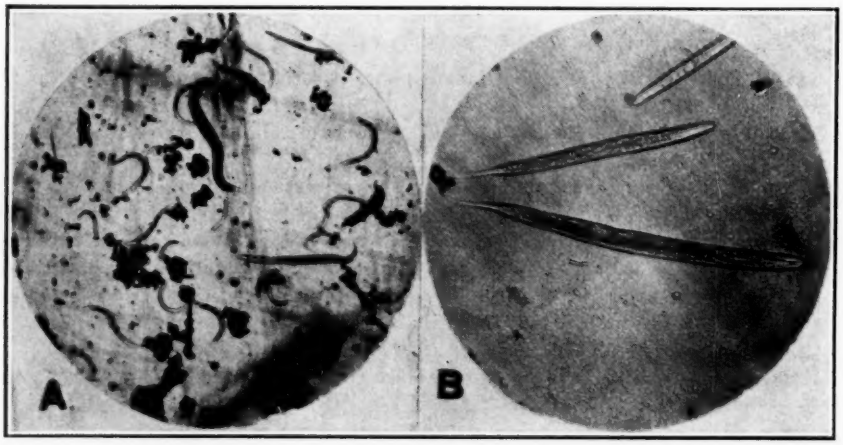

FIG. 1. A. Living nematodes, larvae and adult. ×85. B. Dead larvae of *Rhabditis* sp. ×210.

At first, it was believed that the nematodes promoted a more rapid infection with disease or protected the consumed spores of the pathogenic fungi against the fungicides of insufficiently strong concentration. Later on, however, the nematodes were found in great number in the tissue of live seedlings shortly after germination. This observation furnished strong evidence that the organisms in question acted as primary parasites, although

[1] For general description of nematodes see: S. A. Waksman, Principles of Soil Microbiology, 1932, 2d ed., Baltimore, p. 326–335 and Marcinowski, K. Parasitisch u. semiparasitisch an Pflanzen lebende Nematoden. Arb. K. Biol. Anst. Land u. Forst. 7: 1–192. 1909.

no reinoculation experiments were carried on because of the technical difficulties involved in the isolation of nematodes in pure culture.

The microscopic observations showed that the nematodes are highly resistant to fungicides. Instant death of larvae took place only when they were brought in contact with 2 per cent by volume of H_2SO_4 solution. The animals survived for 5 minutes in 1 per cent H_2SO_4 and in 2 per cent HNO_3, and for more than 15 minutes in 2 per cent solution of formaldehyde and 7 per cent solution of aluminum sulphate.

Pot experiments in the greenhouse with the infested soils, using different fungicides and a variety of coniferous species, have shown that the control of parasites in the nurseries will not be possible in practice with the use of any chemical except 2 per cent sulphuric acid (2 parts of conc. com. acid, sp. grav. 1.8 to 100 parts of water). The latter gave satisfactory results when applied in sufficient quantity to saturate the soil in the entire jar and when the excess was removed by watering. This treatment on granitic sand nearly doubled the amount of available phosphorus, but removed the available nitrogen and most of the available potash. An application of organic matter and mineral fertilizers was absolutely necessary for the proper growth of the seedlings. The common practice of sprinkling on the soil an equivalent amount of acid in a high concentration (about 1 to 7) produced inconsistent and unsatisfactory results.

Although the experiences and observations described are of a purely incidental character, they suggest that some species of nematodes, either directly or indirectly, participate in the destruction of coniferous seedlings during the early period of growth. The writer believes, therefore, that proper attention to these organisms may prevent the sad exeperience of the citrus growers, which is briefly reported by N. A. Cobb[2] as follows: "Our ignorance concerning nematodes in general, and soil-inhabiting nematodes in particular, is well illustrated by the history of the citrus-root parasite *Tylenchus semipenetrans*, which within few months of its discovery in California has been located in such widely separated places as Florida, Spain, Malta, Palestine, and Australia. This series of events is, the writer believes, simply illustrative of the surprises in store whenever the soil-inhabiting nematodes receive at the hands of agricultural scientists the attention they merit."—S. A. WILDE. University of Wisconsin.

[2] Citrus-root nematode. Jour. Agr. Res. 2: p. 217. 1914.

The American Phytopathological Society, in business session December 31, 1935, unanimously approved the action of the Council levying a charge of $1.00 per printed page of all contributions accepted for publication in PHYTOPATHOLOGY on and after January 1, 1936, in accordance with the authority granted at the Atlantic City meeting in December, 1932.

It was voted also to charge to the author the cost of all illustrations in excess of that of 2 full-page halftones, or equivalent, accompanying any one paper. Payment should be made by check or money order to H. A. Edson, Treasurer, Bureau of Plant Industry, Washington, D. C.